Final Technical Report

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Center Name: Southern California Particle Center and Supersite (SCPCS)
Center Director: John R. Froines
Title: Southern California Particle Center and Supersite (SCPCS)
Investigators: John R. Froines[1], Arthur Cho[1], Jon Fukuto[1], William Hinds[1], Antonio Miguel[1], Andre Nel[1], Beate Ritz[1], Robert Schiestl[1], Richard Turco[1], Arthur Winer[1], Rong Chun Yu[1], Edward Avol[2], Kiros Berhane[2], W. James Gauderman[2], Nino Kunzli[2], Robert McConnell[2], John Peters[2], Constantinos Sioutas[2], Dan Stram[2], Yoshito Kumagai[3], Henry Gong[4], William Linn[4], Jack Harkema[5], Michael Kleinman[6], Robert Phalen[6], Fred Lurmann[7], Judith Charles[8], Janet Arey[9], Roger Atkinson[9]
Institutions: [1]University of California–Los Angeles, [2]University of Southern California, [3]University of Tsukuba, [4]Rancho Los Amigos Medical Center, [5]Michigan State University, [6]University of California–Irvine, [7]Sonoma Technology, Inc., [8]University of California–Davis, [9]University of California–Riverside
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RFA: Airborne Particulate Matter (PM) Centers (1999)
Research Category: Particulate Matter

Objective(s) of the Research Project:

Southern California Particle Center and Supersite Overview

The overall objective of the Southern California Particle Center and Supersite (SCPCS) was to bring together outstanding scientists from the University of California–Los Angeles (UCLA), University of Southern California (USC), UC–Riverside, UC–Davis, UC–Irvine, Rancho Los Amigos Medical Center, Sonoma Technology, Michigan State University, and University of Tsukuba, Japan to identify and conduct high priority research to better understand exposure to and effects of airborne particulate matter (PM) and ensure protection of public health. Participating SCPCS faculty represented a wide range of disciplines including toxicology, epidemiology, biostatistics, immunology, pharmacology, medicine, atmospheric sciences, atmospheric and environmental chemistry, exposure assessment, and aerosol science.

The SCPCS developed an integrated approach to address the research needs in exposure, dosimetry, toxicology, and epidemiology of PM that were identified in the U.S. Environmental Protection Agency (EPA) Request for Application (RFA). SCPCS research included interaction and funding from the California Air Resources Board (CARB) and the South Coast Air Quality Management District (AQMD).

The Los Angeles Basin (LAB) is home to more than 14 million individuals who breathe some of the most polluted air in the U.S. The research projects in the SCPCS resulted in a detailed

characterization of PM size, composition, and spatial variability across the LAB, applying measurement, analytical and modeling methodologies. Toxicology investigations were directed toward a fundamental understanding of the mechanisms and the chemical and physical components of PM responsible for health effects. Epidemiologic and human clinical studies provided new information to enhance our ability to detect human health effects from ambient air pollution. Given the continuing poor air quality in Southern California, it is unfortunate that a Center similar to SCPCS was not established years prior to 1999 (the commencement of the SCPCS). The need for this type of Center has not diminished. The SCPCS generated a wide range of projects and produced important findings and data within the themes and hypotheses that guided the overall research effort.

Summary of Findings:

Research Themes and Specific Projects

The principal theme of the SCPCS was: "Mobile Source Pollution and Health Effects." All research within the SCPCS was intended to address specific priorities that had been articulated by the National Research Council (NRC) committee. The second theme that guided the research of the Center was: "Identification of the important physical/chemical characteristics of airborne PM responsible for the adverse health effects associated with PM and co-pollutant exposures." A key feature of the research in the SCPCS was this linkage between exposure assessment/PM characterization and toxicological and human health outcomes. Health studies involved simultaneous physical and chemical characterization of the PM samples.

The SCPCS research programs were developed in three topic areas:

A. Studies emphasizing investigation of the biological mechanisms of PM effects in relation to PM physical and chemical characteristics

B. Studies of emission sources and related adverse health effects

C. Studies of the varying spatial and temporal patterns of ambient PM and co-pollutants and resulting health effects with a particular emphasis on the role of atmospheric chemistry

The studies in the SCPCS were conducted in the context of the secondary theme stated above, and one of the three general research areas. Research areas B and C were complementary and fundamentally linked with that in A. All three focused on the role of organic and metal constituents of PM, in accordance with our primary and secondary research themes. The research mission of the SCPCS was enhanced by the availability of ultrafine, fine, and coarse mobile concentrators which were essential for chemical toxicology, *in vitro* sample collection, *in vivo* studies, and human clinical studies and supported by the efforts of research core groups.

SCPCS Projects

This final report, covering each of the SCPC research themes, is presented as a series of individual reports from the 14 research projects listed below. In addition, there is a report from

the dosimetry core on activities over the six year funding period, listed as project 16 (R827352C016) below.

Topic A: Investigation of the Biological Mechanisms of Particulate Matter (PM) Effects in Relation to PM Physical and Chemical Characteristics:

1. The Chemical Toxicology of Particulate Matter

2. Biological Effects of Diesel Exhaust Particulate and PM *In Vivo* and *In Vitro*

3. Measurement of the "Effective" Surface Area of Ultrafine and Accumulation Mode PM

4. Effects of Exposure to Freeways with Heavy Diesel Traffic and Gasoline Traffic on Asthma Mouse Model

5. Effects of Exposure to Fine and Ultrafine Concentrated Ambient Particles Near a Heavily Trafficked Freeway in Geriatric Rats

Topic B: Studies of Emission Sources and Adverse Health Effects:

6. Relationship Between Ultrafine Particle Size Distribution and Distance From Highways

7. Exposure to Vehicular Pollutants and Respiratory Health

8. Traffic Density and Human Reproductive Health

Topic C: Studies of the Effects of Varying Spatial and Temporal Patterns of Ambient PM and Co-pollutants and Resulting Health Effects with Emphasis on the Role of Atmospheric Chemistry:

9. The Role of Quinones, Aldehydes, Polycyclic Aromatic Hydrocarbons, and Other Atmospheric Transformation Products on Chronic Health Effects in Children: Exposure Assessment

10. A Novel Method for Measurement of Acrolein in Aerosols

11. Off-line Sampling of Exhaled Nitric Oxide in Respiratory Health Surveys

12. Acute Cardiopulmonary Responses to Concentrated Ambient Particulate Matter in Human Volunteers

13. Particle Size Distributions of Polycyclic Aromatic Hydrocarbons in the Los Angles Basin

14. Physical and Chemical Characteristics of PM in the LAB: Source Receptor Study Exposure Assessment

15. Exposure Assessment and Airshed Modeling Applications in Support of SCPC and CHS

16. Report on the Activities of the Dosimetry Core.

The project reports that follow detail the research the SCPCS has undertaken in investigation of these topical areas.

The following definitional/abbreviation convention is adopted throughout this document: F corresponds to airborne fine particulate matter (diameter <2.5 μm); C corresponds to airborne coarse particulate matter (diameter 2.5–10 μm); and UF corresponds to airborne ultrafine particulate matter (in most cases, particle diameter < 0.18 μm). The term accumulation mode is also used for F in some applications. Where the Sioutas concentrator is concerned, F represents the combination of F and UF particles (F + UF).

Topic A: Studies Emphasizing Investigation of the Biological Mechanisms of PM Effects in Relation to PM Physical and Chemical Characteristics

Project 1: The Chemical Toxicology of Particulate Matter (R827352C001)
Investigators: Arthur Cho, John Froines

Objective(s) of the Research Project: The research on chemical toxicology aimed at testing the hypothesis that PM contains reactive chemical species and they, either separately or as a mixture, are responsible for the toxicological phenomena associated with PM. The objective of this project was to characterize PM samples for the chemical properties that likely contribute to their adverse health effects. As the most general mechanism, the chemical basis for the induction of oxidative stress was the focus of the work. Reactive chemical species in PM can be organic and inorganic, and may act through several possible chemical reactions with biological substrates. We focused on two general mechanisms: redox and electrophilic reactions. Redox reactions involve the catalytic reduction of oxygen to reactive oxygen species by components of PM with electrons from biological sources. In the electrophilic reactions, a reactive function in PM reacts with nucleophilic functions in biological systems to form covalent bonds. These bonds are irreversible so that the affected biological molecule is destroyed. The thiol group is a likely target of the two reactions as this group participates in both redox and covalent bond forming reactions. Thiols serve key functions in proteins such as enzymes, transporters and receptors so their modification can result in substantial disruption of cell biochemistry. By characterizing and quantitatively determining the reactivity in a given PM sample with respect to these chemical properties, we intended to be able to predict its potential toxicity.

To test our hypotheses, we developed and continued to develop quantitative assay procedures that can be applied to PM samples to assess their chemical reactivity and potential toxicity as predicted by the considerations above. The development of the assays and their applications are described in the findings section below.

Summary of Findings:

Analytical Procedures

Gas Chromatograpy/Mass Spectrometry (GC/MS) Assay for Quinones (Cho, et al., 2004). The role of quinones in cellular toxicity has long been recognized and their contribution to the toxicity of air pollution was also recognized (Dellinger, et al., 2001; Squadrito, et al., 2001). In an effort to determine the quantities of quinones in PM, a GC/MS based procedure was developed which provided the first quantitative assessment of quinones in ambient air masses. The high sensitivity of the procedure allowed quantitative analysis of small sample sizes, and in the initial report, was applied to a National Institute of Standards and Technology (NIST) standard, diesel exhaust particles and ambient air samples collected in the LAB. The findings of that work are reported in more detail below. A recent report by another group has described a liquid chromatography/mass spectrometry procedure to analyze the same quinones. Their procedure requires larger samples (Valavanidis, et al., 2006) but when applied to ambient samples, the results were consistent with ours.

Dithiothreitol Assay for Redox Activity (Cho, et al., 2005). The redox properties of airborne particulate matter have been recognized for some time (Sagai, et al., 1993; Kumagai, et al., 1997; Dellinger, et al., 2001; Squadrito, et al., 2001) and a role for redox activity in the cellular effects of PM has been proposed (Kumagai, et al., 1997; Li, et al., 2000; Squadrito, et al., 2001; Hirano, et al., 2003; Li, et al., 2004; Valavanidis, et al., 2005). Our dithiothreitol (DTT) assay was developed to assess the redox properties of a given PM sample in a quantitative manner and is based on the ability of the sample to catalyze the electron transfer between DTT and oxygen. The procedure determines the rate at which DTT is consumed, due to the oxidation of DTT as it transfers electrons to oxygen in a process catalyzed by the test sample. We have shown that redox active quinones such as the naphthoquinones and phenanthroquinone, diesel exhaust particles and the particulate and volatile fractions of ambient air exhibit this property. When applied to ambient air samples, the rate of DTT consumption is a function of the sample as a whole, so that an integrated metric of exposure to this redox activity is obtained. The specific compound(s) that catalyze the reaction are not determined by this assay. In its initial evaluation, ambient air samples were collected in the LAB with a VACES concentrator and a liquid impactor and the samples assayed with this procedure.

Ascorbate-Salicylate Based Assay for Metal Based Redox Activity. The apparent selectivity of the DTT assay for organic compound based redox activity and the need for assessment of metal based redox properties led us to modify an assay developed by Kelly measuring the consumption of ascorbic acid (Mudway, et al., 2004). By including salicylate in the reaction mixture, the transition metals in the test sample catalyze the Fenton reaction, in which hydrogen peroxide generated by the reduction of oxygen is converted to hydroxyl radical. This highly reactive oxygen species will rapidly hydroxylate salicylate to isomers of dihydroxybenzoic acid (DHBA) which is readily measured by high-performance liquid chromatography (HPLC) and an electrochemical detector. The use of this latter reaction to determine hydrogen peroxide and cellular oxidative stress is well established (e.g., Coudray and Favier, 2000; Liu, et al., 2003). We have applied it to ambient PM samples, collected with Teflon filters or with the biosampler and found measurable activities. This activity, expressed as nmoles of total DHBA formed per minute per microgram of sample, is completely blocked by the metal chelator, diethylene triamine pentaacetic acid (DTPA), consistent with the participation of metal ions in the reaction. The assay is currently being examined as a complementary analysis to the DTT assay that will

detect metal based redox activity, and has been applied in a comparative study of samples collected on the 110 freeway and on samples collected in the Caldecott Tunnel (see below).

GAPDH Based Assay for Electrophilic Properties in Samples. Electrophilic reactions represent an important chemical mechanism, in addition to generation of reactive oxygen species, by which PM and its reactive constituents could cause adverse cellular effects. However the electrophilic properties of airborne pollution have not yet been characterized. During our related work on the mechanisms of quinone toxicity in yeast, the thiol based enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was found to be a key cellular target (Rodriguez, et al., 2004). Our subsequent studies revealed two mechanisms of GAPDH inhibition that can be carried out by various quinones. The first mechanism is dependent on reactive oxygen species (ROS) generation. The second mechanism is oxygen independent and involves covalent interactions between the catalytic thiol in GAPDH and electrophilic quinones (Rodriguez, et al., 2005). Electrophilic inactivation is characterized by two parameters: a rate constant for inactivation ($k_{inactivation}$) and an affinity parameter, K_i, which reflects the concentration of inhibitor needed to decrease the overall rate by 50% under a standard set of conditions. This assay has been applied to two test quinones that are present in atmospheric samples, and a diesel exhaust extract (see below). Currently, a protocol for the assay that is applicable to samples from different ambient collection devices is being developed.

Application of Assays to Ambient PM Samples

As part of overall SCPCS activities, a variety of ambient PM samples were collected at various locations within the LAB. Selected samples were then transferred to our laboratory to be tested in our assays for quinones and chemical reactivity. The SCPCS conducted several sampling campaigns at sites heavily influenced by traffic, including freeway and tunnel sites. Seasonal differences in PM characteristics were also explored.

Application of DTT Assay to LAB Ambient Air Samples

Ambient air samples were collected with a VACES concentrator and a liquid impactor and DTT activity was assessed. Physical correlates of activity were evaluated to identify chemical species that might be involved and to compare the C, F, and UF fractions for their redox activity (Cho, et al., 2005). The study found:

1. The DTT-based redox activity did not correlate with any of the inorganic species measured, including metals (elemental and ions), sulfate and nitrate. The highest DTT activity was found in the UF fraction, which has the highest content of organic species.

2. The activities correlated with organic carbon (r^2=0.53), elemental carbon (r^2 = 0.79) and polycyclic aromatic hydrocarbon (PAH) levels in the PM sample. The highest correlation was with benzo[ghi]pyrene (BgP) (r^2 = 0.82). BgP is regarded as a marker for vehicular combustion emissions, especially from gasoline motor vehicles (Miguel, et al., 1998).

3. The DTT activity in ambient samples was found to correlate with the ability of a given PM sample to induce the stress protein, hemeoxygenase-1 in tissue culture cells (Li, et al., 2003),

so that the UF fraction was associated with chemical and biological activity. These findings support the hypotheses of the project that toxic properties of PM may be attributable, in part, to the organic carbon compounds with which the assay findings were most highly correlated.

4. The observed redox activity was normalized to mass to enable comparison of the potency of samples from each site, and normalized to volume of air to enable comparison of exposure to reactive chemical species. When normalized to mass, Claremont, an area east and downwind from central Los Angeles, exhibited the highest activity. Claremont is considered a "receptor" site because it receives an air mass that has been subjected to photochemical reactions during its movement across the basin. Oxidation by photochemical reactions could account for its higher activity, since redox active compounds such as quinones are photo-oxidation products.

5. When the activity is normalized to volume of air, the area adjacent to the USC campus had the highest exposure levels, due to the high particle count.

Redox Properties of PM From the 110 Freeway

The 110 Freeway was used predominantly by small vehicle traffic in the downtown area of Los Angeles. Two collection campaigns were conducted, one during the summer of 2004 and a second in the winter of 2005. The redox activity of both sets of samples was determined by the DTT-based assay, and the winter samples were additionally assayed by both the DTT and ascorbate-dihydroxybenzoate assay procedures, in a pilot application of this assay to ambient PM samples. The results are summarized in Table 1, with activity expressed per mass in the first two columns and DTT activity expressed per volume of air in the last column.

Table 1. Redox Activity of PM (Summer 2004 and Winter 2005)

	DTT $nmol*min^{-1}*mg^{-1}$	**DHBA** $nmol*min^{-1}*mg^{-1}$	**PM Mass** $mg*m^{-3}$	**DTT Activity** $nmol*min^{-1}*m^{-3}$
110 Summer C	0.008	ND	123.300	0.9864
110 Summer F	0.031	ND	33.900	1.0509
110 Summer UF	0.056	ND	9.600	0.5376
110 Winter C	0.017	0.024	10.600	0.1802
110 Winter F	0.025	0.018	13.000	0.325
110 Winter UF	0.042	0.045	14.700	0.6174

ND: Not Determined
C: Coarse; F: Fine; UF: Ultrafine

The DTT activity per mass is the highest in the UF fraction in both the summer and winter freeway samples. This is consistent with samples from non-roadway source and receptor sites in the LAB (above). When the 110 Freeway samples were compared for exposure to redox activity per volume, the C and F contributed the greatest exposure concern in the summer whereas the

UF contributed the highest exposure levels in the winter. This reflects a shift in the size distribution of PM with season.

DHBA formation was also the highest in the UF fraction, but activity was also clearly present in C samples, consistent with higher metal concentrations in C. It is possible that DHBA formation by UFs may be reflective of hydrogen peroxide levels rather than metal content.

Caldecott Tunnel Study

The assays for chemical reactivity were applied in a study of the air mass in the Caldecott Tunnel, located in Northern California. This tunnel provides access to an enclosed environment of vehicular traffic. The Caldecott has two bores, one of which is used by gasoline and diesel vehicles (CB1, mixed) and a second which is used exclusively by gasoline vehicles (CB2, gasoline). Liquid impinger samples were collected in the summer and winter seasons and assayed for redox activities. The concentrations of PM in the tunnels were very high, roughly an order of magnitude greater than at the 110 Freeway. Tunnel concentrations of C were higher in the summer, while F and UF levels in the tunnel remained more consistent between seasons.

Subsequent to the collections, the glass containers used for the winter samples were found to produce a redox artifact of ascorbate consumption because of variable but low levels of trace metals that contribute to the blank. Assay findings are presented in Table 2 for summer 2004 samples.

Table 2. Redox Properties of PM From the Caldecott Tunnel (Summer 2004)

	DTT $nmol*min^{-1}*mg^{-1}$	**Ascorbate** $nmol*min^{-1}*mg^{-1}$	**DHBA Total** $nmol*min^{-1}*mg^{-1}$	**PM Mass** $mg*m^{-3}$	**DTT Activity** $nmol*min^{-1}*m^{-3}$
Bore 1 Coarse	0.019	0.051	0.0050	988.000	18.772
Bore 1 Fine	0.068	0.041	0.0039	644.000	43.792
Bore 1 UF	0.111	0.117	0.0002	230.000	25.530
Bore 2 Coarse	0.032	0.061	0.0052	581.000	18.592
Bore 2 Fine	0.075	0.081	0.0070	477.000	35.775
Bore 2 UF	0.172	0.115	0.0041	130.000	22.360

UF: Ultrafine

The UF possessed higher DTT activity relative to the F and C, consistent with the findings at the 110 Freeway and non-roadway sites. Interestingly, we found somewhat higher DTT activity in the UFs collected in bore 2, which is limited to light duty traffic (primarily gasoline), relative to samples from bore 1. We compared DTT results to chemical composition analyses of the samples; neither elemental nor organic carbon levels correlated well with DTT activity in this set of samples.

When expressed per mass, the activities for the 110 and Caldecott samples were comparable, but the large mass found in the tunnels resulted in much greater total redox activity in the air mass. Tunnel commuters may have high overall exposure to potentially toxic materials during tunnel transit.

Activity in the ascorbate assay was essentially in agreement with DTT results in the summer tunnel samples. We consider that these assays may provide some redundancy in measuring the levels of redox activity. However, it is important to note that the results of the two assays have not been congruent in all sample sets tested. The DHBA data suggest an inverse association with particle size in the tunnel bore bearing mixed heavy and light duty traffic, consistent with the greater level of metals expected in C vs. UF. We found somewhat higher overall DHBA production in the samples from the gasoline only tunnel, although the association with greater particle size was not as pronounced.

Quinone Concentrations in Particle and Volatile Fractions

Caldecott Tunnel. Filter and volatile air samples were collected on Teflon filters ($PM_{2.5}$) with XAD resin beds below the filters, during the 2004 Caldecott campaign. The filters and XAD beds were extracted with dichloromethane and the concentrations of four quinones determined by GC/MS (Cho, et al., 2004) (Figure 1). The results from this analysis show that all four of the quinones were present with high levels of 9,10-AQ in the $PM_{2.5}$ fraction and high levels of 1,4-NQ in the volatile, XAD, fraction. No clear seasonal pattern was apparent. The substantial day to day variability is shown by the standard deviations observed for 9,10-AQ in particular. This variability limits the ability to evaluate differences in levels for their significance.

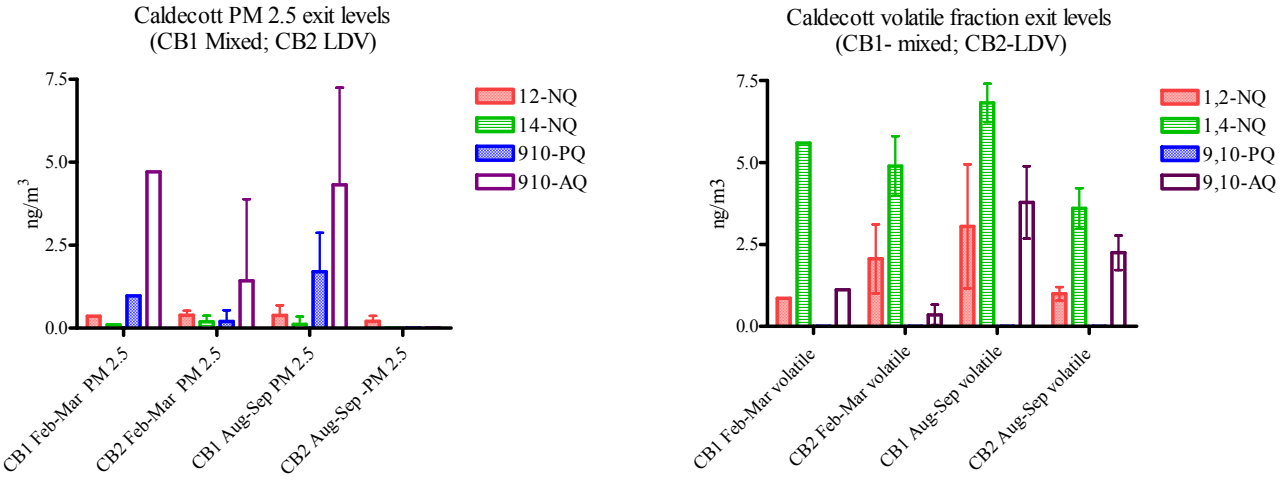

Figure 1. Quinone Concentrations in the Caldecott Tunnel

1. The wide variability in the quinone content for a given day was such that seasonal differences were not significant. Differences in the distribution of the quinones between particle and vapor phases were noted, with the three ring quinone, 9,10-phenanthroquinone (9,10-PQ) found mostly in the particle fraction while the naphthoquinones, 1,2- and 1,4-naphthoquinone (1,2- and 1,4-NQ) were found in the vapor phase at levels substantially greater than 9,10-PQ

2. The consistently high concentration of the naphthoquinones in the volatile fraction reflects the high concentration of the parent aromatic hydrocarbon in exhaust and in ambient air

(Fraser, et al., 1998). Their presence in the tunnel would suggest that these quinones are formed from combustion. Their levels did not appear to differ between the two bores

3. There appears to be a trend toward higher levels of the three ring quinones, 9,10-PQ and 9, 10-AQ in the particle phase of bore 1, with mixed traffic than bore 2. This could reflect the presence of the heavy-duty vehicles (HDVs) in bore 1

Other Studies

The Redox Properties of Diesel Exhaust (Pan et al., 2004). Diesel exhaust particles, obtained from the laboratories of Professor Sagai in Tsukuba, Japan, have been used in many of our assay procedures (Li et al., 2003; Cho et al., 2004; Cho et al., 2005) as a standard. This sample is available in large quantity and diesel exhaust particles (DEP) are directly relevant to the urban aerosols of major interest to the SCPCS. This material was examined for its redox properties using DTT and ascorbate as electron sources. In the presence of either of these reducing agents, whole particle suspensions were able to reduce oxygen to superoxide or peroxide in a catalytic process that was dependent on the particle concentration. To characterize the nature of the contributing species, the particles were subsequently extracted with an organic solvent (dichloromethane) and dilute acid (1 M HCl) to remove organic and metal species, respectively, that contribute to the overall activity. The results of this study showed that while the dichloromethane extract contained species that exhibit redox activity, a considerable proportion of the original activity remained in the particle residue after extraction. Furthermore, when the extract and the particles were combined to reconstitute the original preparation, the overall activity was greater than that found before extraction. The extraction with dilute acid also reduced the activity of the original particles, but a reconstitution experiment was not performed. These observations indicate that while a fraction of the redox activity associated with diesel exhaust particles is extractable by both organic solvent and acid, most of the redox activity is associated with the particles themselves. Furthermore, extraction of DEP with organic solvents appears to alter the chemical properties of the particles such that their reactivity increases, indicating a complex interaction between particle structure and extractable components.

Using electron paramagnetic resonance spectrometry, DEP were shown to have inherent paramagnetic properties. This suggests unpaired electrons that may be important to the ability to catalyze the formation of reactive oxygen species and at least partially responsible for their toxicity. The paramagnetic species identified was resistant to extraction, as described above.

Electrophilic Properties of PM and Related Quinones. Our assay for inhibition of GAPDH was performed on two quinones that have been detected in atmospheric samples, and a diesel exhaust extract to compare the mechanisms by which these compounds inhibit the enzyme (Rodriguez, et al., 2005). The redox active 9,10-PQ inactivates the enzyme by both oxygen dependent and independent mechanisms whereas 1,4-BQ, a pure electrophile in biological systems, inactivates the enzyme by an electrophilic, oxygen independent mechanism. The rate constants for affinity (K_i) and inactivation ($k_{inactivation}$) for the two quinones, determined under anaerobic conditions, are shown in Table 3, together with the $k_{inactivation}$ determined for a diesel exhaust extract that we have used in all of our experiments as a "standard." Experiments with DEP extracts showed an analogous time dependent inactivation that could be prevented by coincubation with high

concentrations of alternate thiols, consistent with covalent attachment to the enzyme thiol. These results are the first to demonstrate the electrophilic nature of DEP constituents and indicate that with this source of PM, the induction of cellular stress by pathways other than reactive oxygen generation is possible.

Table 3. GAPDH Inactivation

Compound	**Ki (μM)**	**$k_{inactivation}$**
9,10-PQ	22.54	1.47 ($\mu M^{-1}*min^{-1}$)
1,4-BQ	.67	0.57 ($\mu M^{-1}*min^{-1}$)
DEP extract	ND	.0028 $(\mu g/mL)^{-1}*min^{-1}$

Summary

The objective of this project was to characterize PM samples for the chemical properties that likely contribute to their adverse health effects. The project developed assays designed to assess these chemical properties on a quantitative basis. The assays were based on known redox and electrophilic properties of quinones whose presence in PM had been reported in qualitative terms (Kumagai, et al., 1995; Koeber, et al., 1999). Our assays used quinones as standards in the development process, and the assays were developed for samples of particulate material on filters, in aqueous suspensions or as extracts of XAD resins containing volatile substances. Key findings from this project include:

1. We determined the concentration of four quinones found in both particulate and volatile fractions of ambient air samples and demonstrated that the assay can be used to determine changes in these quinones as an air mass moves across the LAB. The results are consistent with the notion that at least two of the quinones, 1,4-NQ and 9,10-PQ are formed by photochemical processes in the LAB.

2. We have determined the redox activity of air samples collected at different sites in the LAB and found that while the activity per mass does not vary over a wide range, the particle concentration variability is such that exposure can be vastly different at different sites and in different seasons. UF in general have a higher activity per mass, while the greater mass concentration of F often renders this fraction responsible for the greatest exposure to material that is active in the DTT assay.

3. We have found that samples of PM from roadways possess redox activity. In a study of the redox properties of diesel exhaust particles, we have observed that the organic and acid extractable materials present in the particles posses redox activity but that the particles retain significant activity after extraction.

4. We have obtained preliminary evidence to support the notion that particles and their constituents exhibit electrophilic properties, as evidenced by the inhibition of the nucleophilic enzyme, GAPDH.

Our studies demonstrate that UFs have greater potency in redox cycling assays, and that this potency may be attributable to organic chemicals associated with combustion processes. Chemical properties that derive from the particle structure, not only the constituent compounds, are relevant and our DTT assay reflects the behavior of whole particles not limited to any specific constituent. Other research in the SCPCS found that cell biological effects of PM that are related to oxidative stress and mitochondrial damage are greater with UF, indicating that activity in our chemical assays may predict biological toxicity of PM samples.

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Project 2: Pro-inflammatory and the Pro-oxidative Effects of Diesel Exhaust Particulate *in Vivo* and *in Vitro* (R827352C002)
Investigators: Andre Nel, Ning Li, Constantinos Sioutas, Arthur Cho, John Froines

Objective(s) of the Research Project: Research in this project has increased our understanding of the mechanisms by which PM induce adverse health effects. Progress has been made in understanding the oxidative stress pathways by which diesel exhaust particulate (DEP) and ambient PM mediate injury, and has also helped to elucidate the adjuvant effects of DEP in asthma. We will address research findings and conclusions from our work according to three project aims.

Summary of Findings:

Aim 1: To Elucidate the Role of Reactive Oxygen Species (ROS) and Inflammation in PM-Induced Adverse Health Effects *In Vitro* and *In Vivo*

A potential mechanistic link between PM exposures and inflammation involves the generation of ROS and oxidative stress (Li, et al., 2003; Nel, 2005). A number of our studies, described in more detail below have demonstrated that ambient PM and DEP induce ROS production in target cells such as macrophages and bronchial epithelial cells (Li, et al., 2003a; Nel, 2005; Li, et al., 2002a; Li, et al., 2002b; Hiura, et al., 1999; Li, et al., 2003b). DEP were used in our studies as a convenient model for vehicular UF, which are a common component of ambient aerosols in urban areas.

We performed a series of in vitro and in vivo experiments to explore the link between ROS production, oxidative stress and inflammatory tissue injury (Li, et al., 2003a; Nel, 2005; Mingi, et al., 2003). The findings of the studies form the basis for the development of a hierarchical oxidative stress model (Figure 2). The model posits that at lower levels of oxidative stress (Tier 1), there is an induction of phase II enzymes regulated by a genetic response pathway that involves the transcription factor, Nrf2 (Li, et al., 2003a; Nel, 2005; Li, et al., 2002b; Gilmour, et al., 2006). Nrf2 drives the antioxidant response element (ARE) in the promoter of phase II response genes, leading to the expression of antioxidant and detoxification enzymes (Li, et al., 2004). Treatment of target cells in vitro with DEP, organic DEP extracts or ambient UF induced the expression of heme oxygenase 1 (HO-1), glutathione-S- transferase (GST), NADPH quinone

oxidoreductase (NQO1), catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glucoronosyltransferase (UGT) (Li, et al., 2004; Li, et al., 2000). These phase II enzymes protect against oxidative stress injury (Tiers 2 and 3), and a reduced Tier 1 defense could therefore promote PM susceptibility (Li, et al., 2003a; Nel, 2005). In humans, reduced defenses against oxidative stress can result from phase II enzyme polymorphisms, e.g., the GST M1 null genotype which predisposes to the development of asthma and enhanced sensitization to common environmental allergens during nasal DEP challenge (Li, et al., 2003a). Conversely, induction of a phase II response may be a key factor in adaptation to a polluted environment, and may explain why persistent inflammatory changes in the lung are not observed after repeated exposure to low concentrated ambient particles (CAPs) levels. Some phase II enzymes, such as HO-1, exert anti-inflammatory effects based on their ability to interfere with pro-inflammatory response pathways (Li, et al., 2000; Li and Nel, 2006).

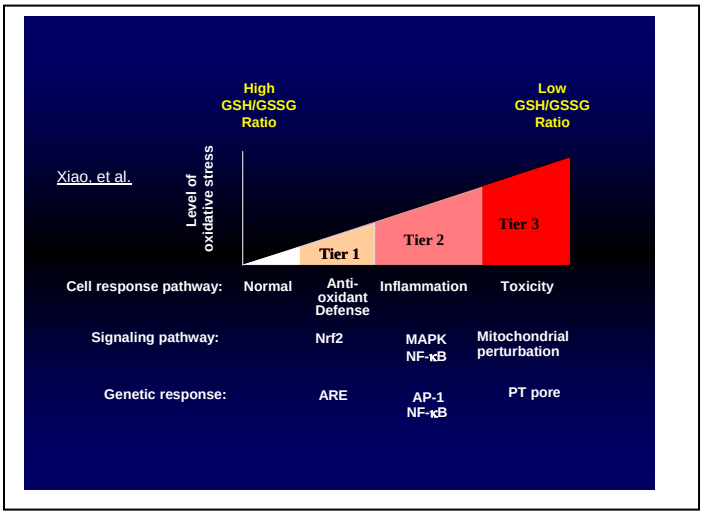

Figure 2. Hierarchical Oxidative Stress Model

If Tier 1 protection fails, our model proposes that further increase in oxidative stress generates pro-inflammatory responses (Tier 2) or cytotoxic effects (Tier 3) depending upon the level of insult and response capability of the exposed cells (Figure 2). Tier 2 responses are linked to the activation of intracellular signaling pathways which impact cytokine and chemokine production (Li, et al., 2003a). An example is activation of the MAP kinase cascade (Li, et al., 2003a). This cascade is responsible for the expression and activation of AP-1 transcription factors (e.g., c-Jun and C-Fos), which in turn are responsible for the expression of a variety of pro-inflammatory genes, including those encoding for cytokines, chemokines and adhesion molecules. Tier 3 responses in our model involve mitochondrial perturbation by pro-oxidative chemicals (Hiura, et al., 1999; Li, et al., 2003b; Hiura et al., 2000; Xia, et al., 2004). Although the in vivo significance of the mitochondrial pathway is uncertain, we have demonstrated in tissue culture cells that PM-induced interference in one electron transfers in the mitochondrial inner membrane and perturbation of the mitochondrial permeability transition pore (PTP) can contribute to superoxide generation and the induction of cellular apoptosis (Hiura, et al., 1999; Li, et al., 2003b; Hiura et al., 2000; Xia, et al., 2004). These effects can be mimicked by organic extracts made from DEP as well as redox cycling quinones and functionalized aromatic hydrocarbons present in the DEP particles (Li, et al., 2002a; Li, et al., 2000). Each tier of oxidative stress is

sensitive to the effects of NAC (Li, et al., 2002b; Hiura, et al., 1999; Whitekus, et al., 2002; Xia, et al., 2004).

The principles of a hierarchical oxidative stress response were tested in macrophages and epithelial cell cultures exposed to ambient UF, DEP extracts, or fractionated DEP extracts (Li, et al., 2002a). At the lowest tier of oxidative stress (Tier 1), the expression of catalase, SOD, and HO-1 indicates the involvement of Nrf2-regulated enzymes that can suppress inflammation through their antioxidant activities (Li, et al., 2003a; Li, et al., 2004). This finding was extended by showing that particulate pollutants increase the accumulation of Nrf2 in the nucleus and activate the ARE (Li, et al., 2004). Interestingly, the buildup of Nrf2 in the nucleus is dependent on a prolongation of protein half-life by interference in proteosomal degradation (Li, et al., 2004). Activation of the ERK, p38 and Jun kinase cascades was confirmed by phosphor-proteome analysis (Wang, et al., 2005). To further substantiate the findings, related experiments are now being conducted in vivo, using BAL fluid and lung tissue from PM-exposed animals to find in vivo biomarkers of oxidative stress. These markers could be useful to identify the subsets of the human population susceptible to PM exposure.

While there is still considerable debate about which particle components are responsible for the pro-oxidative and pro-inflammatory effects associated with PM, our work adds to accumulating evidence that transition metals, such as copper, vanadium, chromium, nickel, cobalt and iron, as well as aromatic and polar organic substances play a role in ROS production (Li, et al., 2003a; Li, et al., 2000). The particle backbone could play an important role in acting as a template for single electron transfers reactions, including electron transfer to molecular dioxygen (Figure 3). This could involve redox cycling reactions, as demonstrated by the ability of ambient PM samples to generate superoxide in the presence of DTT (Li, et al., 2003b). DTT oxidation can be assessed by a colorimetric reaction to assay for the content of redox cycling chemicals in urban PM samples (Li, et al., 2003b). In addition, biologically catalyzed oxidation-reduction reactions in the cellular interior, as well as interference in one electron transfers in the mitochondrial inner membrane, contribute to ROS generation (Xia, et al., 2004). In addition to the ability of ROS to damage cellular proteins, DNA and cell membranes, electrophilic PM chemicals such as the quinones can modify cellular proteins by Michael acceptor reactions (Li, et al., 2004). It is likely that this type of reaction leads to Nrf2 release to the nucleus by the covalent modification of its cytosolic chaperone, Keap-I (Li, et al., 2004). The covalent modification of intracellular and tissue proteins was also confirmed by studying their tyrosylation and carbonylation after in vivo exposure to diesel exhaust particulate (Whitekus, et al., 2002).

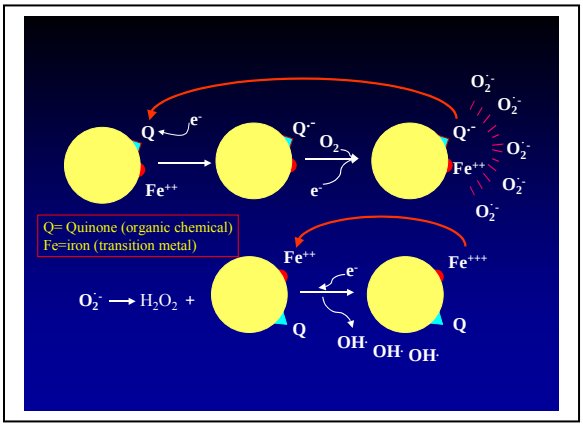

Figure 3. Particle-Induced ROS Production by Key Chemical Components

In experiments to characterize the redox cycling chemicals present in PM, silica gel chromatography was used to fractionate organic DEP extracts (Li, et al., 2002a; Li, et al., 2003b; Li, et al., 2000; Xia, et al., 2004). Aliphatic, aromatic and polar chemical fractions were eluted by increasingly polar solvents and tested for reactivity in the DTT assay. The quinone-enriched polar material was more active than the polycyclic aromatic hydrocarbon (PAH)-enriched aromatic fraction. Glutathione depletion in epithelial cells and macrophages is associated with the exposure to DEP extracts and with activity in the DTT assay (Li, et al., 2002a; Li, et al., 2003b; Li, et al., 2000; Xia, et al., 2004). The aliphatic fraction was inactive in these assays.

The relationship between the organic chemical composition and the redox cycling potential of PM that had been noted for diesel particles was confirmed in a study in which UF were compared to C and F collected in the LAB (Li, et al., 2003b). UF were more active than C and F in the DTT assay, and were also more prone to generate oxidative stress in macrophages and epithelial cells (Li, et al., 2003b). Both the *in vitro* and cellular responses showed an excellent correlation with the PAH content of UF (Nel, 2005; Li, et al., 2003b). Another important observation in this study was the ability of UF to lodge in and disrupt the mitochondrial architecture (Li, et al., 2003b). This finding is related to cellular apoptosis and apo-necrosis by a pathway that requires opening of the mitochondrial permeability transition pore (PTP) (Hiura, et al., 2000; Xia, et al., 2004). Functional effects on the PTP and inability to sustain one electron transductions in the mitochondrial inner membrane was confirmed in isolated mitochondrial preparations through the use of calcium-dependent swelling, calcium retention capacity and dissipation of the mitochondrial membrane potential (Xia, et al., 2004). Moreover, UF particle effects could be reproduced by polar and aromatic chemicals fractionated from DEP, while commercial polystyrene nanoparticles were inactive (Xia, et al., 2004). These data demonstrate differential particle toxicity associated with particle size, composition, and subcellular localization.

<u>Aim 2: To Develop a Murine Model for Asthma to Explain the Adjuvant Effects of DEP on Ovalbumin (OVA)-Induced Allergic Inflammation and Airway Hyperreactivity (AHR)</u>

The asthma studies were premised on findings that DEP enhance allergen-specific IgE and TH2 cytokine production in humans and animals (Li, et al., 2003a; Nel, 2005). We demonstrated that aerosolized DEP can enhance OVA-specific IgE production in a murine inhalation model (Whitekus, et al., 2002). The adjuvant effect of DEP could be suppressed by NAC administration (Whitekus, et al., 2002). While adequate for upregulating IgE production, an important limitation of this model was the inability of DEP to enhance AHR. This likely reflects the fact that oxidizing chemicals lead to efficient IgE gene rearrangement in deposition hotspots (Li, et al., 2003a), but exposure did not exceed the threshold of airway inflammation that is required for AHR (Li, et al., 2003a). DEP-induced AHR has now been achieved through modification of the classical mouse OVA sensitization model, in which sensitization is achieved

by intraperitoneal administration of OVA. We developed two new protocols for OVA sensitization (Mingi, et al., 2003). In the low grade sensitization protocol, BALB/c mice received intraperitoneal OVA without alum, followed by challenge with aerosolized OVA ± DEP two weeks later. In the post-challenge model, DEP was delivered to classically sensitized animals a few days after the OVA challenge. Under both conditions, DEP enhanced airway inflammation to the point of exceeding the AHR threshold (Mingi, et al., 2003). Since these data suggest that low grade airway inflammation is required to elicit AHR, nebulized DEP was administered to mice which have been genetically engineered to overexpress IL-5. These animals exhibit constitutive airway inflammation, and responded to DEP inhalation with increased airway inflammation and AHR (Mingi, et al., 2003).

To demonstrate the role of oxidative stress in the adjuvant effects of DEP in allergic inflammation, we performed an animal study in which mice were sensitized to OVA and co-challenged with aerosolized DEP daily for ten days (Whitekus, et al., 2002). The thiol antioxidants, N-acetylcysteine (NAC) and bucillamine (BUC) were administered intraperitoneally during sensitization. NAC and BUC effectively inhibited the adjuvant effects of DEP in the induction of OVA-specific IgE and IgG1 production (Whitekus, et al., 2002). Furthermore, NAC and BUC prevented the generation of lipid peroxidation and protein carbonylation, an oxidative modification of protein structure, in the lungs of OVA- plus DEP-exposed animals. These findings indicate that NAC and BUC are capable of preventing the adjuvant effects of inhaled DEP and suggest that oxidative stress is a key mechanistic component in the adjuvant effect of DEP. Antioxidant treatment strategies may therefore serve to alleviate allergic inflammation and may provide a rational basis for treating the contribution of particulate matter to asthmatic disease.

Aim 3: Dosimetry and Distribution of Particles

Health effects of exposure to PM are likely to be proportional to the PM dose to critical cells and organs. Tissue dose is influenced by the proportion of inhaled particles that are retained in the lung with each breath. The proposed hierarchical oxidative stress response that occurs in PM target cells has been discussed above. A frequently asked question is how the experimental in vitro DEP concentrations that span the three tiers of oxidative stress (e.g., 1–100 μg/ml in macrophages) can be understood in terms of tissue concentrations that are achieved in the lung during real-life exposures. One method to reconcile doses used in vitro with in vivo exposures, is to convert ambient PM levels, measured in $\mu g/m^3$, to a dose that is deposited on a planar surface and then to compare that to the calculated dose of the DEP that are deposited on a planar tissue culture surface (Li, et al., 2003a). Our calculations resulted in a planar concentration of 0.2–20 $\mu g/cm^2$ on the tissue culture dish. This concentration was compared to a theoretical in vivo deposition dose that would occur in the nasopharyngeal (NPR), tracheobronchial (TBR), and alveolar (AVR) regions of the respiratory tract of an adult person exposed to $PM_{2.5}$ in Rubidoux, California (Li, et al., 2003a). After correction for parameters such as airway anatomy, nasal breathing, high rates of deposition at bifurcation points, and uneven airflow due to airway obstruction in asthma or chronic obstructive pulmonary disease (COPD), the calculated deposition values for the NPR, TBR and AVR were 204, 2.3 and 0.05 $\mu g/cm^2$, respectively, for the Rubidoux scenario. The calculations showed that it is possible to achieve doses in the nose

and TBR from ambient exposures that are responsible for the in vitro induction of antioxidant, pro-inflammatory and cytotoxic responses.

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Project 3: Measurement of the "Effective" Surface Area of Ultrafine and Accumulation Mode PM (Pilot Project) (R827352C003)
Investigators: Sheldon Friedlander, Constantinos Sioutas

Objective(s) of the Research Project: The ultrafine particle size range ($dp < 0.1$ μm) of the atmospheric aerosol is composed of both primary and secondary particulate matter. The primary component, emitted directly from sources, often includes agglomerates of 10 to 50 nm particles. (Note that the term "primary" in this context differs from its use to designate the individual particles that compose aerosol aggregate structures.) The secondary component is composed of particulate matter formed in the atmosphere, including sulfuric acid and sulfates, and organic reaction products of low volatility. Particles that form in the atmosphere tend to evaporate in the electron microscope, our principal observational method. Animal studies indicate that freshly formed agglomerate structures may have adverse health effects (Warheit, et al., 1990). Thus it is important to be able to characterize this component of the atmospheric aerosol.

With support from other sources, our Laboratory developed novel methods for the sampling and analysis of ultrafine atmospheric agglomerates. We applied our technology in a collaborative study with Dr. Constantinos Sioutas. Our goal was to determine whether the condensation and evaporation processes that precede aerosol concentration in the VACES alter the structure of agglomerates in the ultrafine particle size range. In the set of measurements described in methodology below, we found that the agglomerates were concentrated without substantial changes in their structure. The results of our study were described in the publication cited below:

Kim S, Jaques PA, Chang M, Barone T, Xiong C, Friedlander SK, Sioutas C. Versatile Aerosol Concentration Enrichment System (VACES) for simultaneous *in vivo* and *in vitro* evaluation of toxic effects of ultrafine, fine and coarse ambient particles. Part II: Field evaluation. *Journal of Aerosol Science* 2001;32(11):1299-1314.

Summary of Findings:

Methodology

Atmospheric ultrafine particles and those concentrated by the VACES were sampled using the low-pressure impactor (LPI) at the UCLA campus, in west Los Angeles. Measurements for the ambient air and concentrated particles from the VACES were made within minutes of each other. Concentrated ultrafine aerosols generated by the VACES were sampled after they were dried by diffusion. The LPI is an eight-stage single jet impactor equipped with a critical orifice that maintains a flow rate of 1 L/min under the appropriate pressure drop (Hering, et al., 1978; 1979). The stages have 50% efficiency cutoffs for aerodynamic diameters of 4.0, 2.0, 1.0, 0.5, 0.26, 0.11, 0.075, and 0.05 μm for stages one to eight, respectively. The particles were collected on a nickel transmission electron microscope (TEM) grid. To minimize the effects of particle bounce, only one stage at a time had a grid attached for sampling; the grid was secured at the center of a 25 mm diameter glass stage, while the other glass stages were coated with apiezon grease to trap the larger particles. Air was drawn through the impactor by a vacuum pump for 5 minutes per stage for aerosols coming from the concentrator and 10 minutes for the ambient air samples. Agglomerates were collected on LPI stages 7 and 8, which have aerodynamic diameter ranges of 0.075–0.11 μm and 0.05–0.075 μm, respectively. Transmission Electron Microscope (TEM) photomicrographs of the grids were taken using a JEOL 100CX and 2000FX TEM at a magnification of 10^5. To compare the morphologies of the atmospheric agglomerates and those concentrated by the VACES, we made use of fractal concepts. The fractal characteristics determine agglomerate transport and deposition from the atmosphere and in the lung. They may also affect the interaction of agglomerates with cellular surfaces. More details on fractal analysis conducted in our Laboratory can be found in Xiong (2000).

Experiments and computer simulations have shown that fractal concepts can often be applied to agglomerates of nanometer primary particles (Forrest and Witten, 1979; Witten and Sander, 1981). Such agglomerates can be described by the following relationship (Weber, et al., 1995):

$$N_p = A\left(\frac{R_g}{R_o}\right)^{D_f} \quad (1)$$

where D_f is the fractal dimension, N_p is the number of primary particles in the agglomerate, A is the fractal pre-factor, R_o is the average primary particle radius and R_g is the radius of gyration. The radius of gyration is defined by the expression:

$$R_g = [(1/M)\Sigma(m_i r_i^2)]^{1/2} \quad (2)$$

where m_i is the mass of the i^{th} primary particle, M is the total mass given as Σm_i, and r_i is the distance of the i^{th} primary particle from the center of mass. Values of D_f and A for agglomerates sampled from ambient air and from the concentrator exit air were obtained from a log-log plot of the number of primary particles as a function of distance from the center of mass to the edge of the agglomerate. The fractal dimension was determined from the slope of this diagram, and the prefactor from the intercept of the line representing the least squares fit.

Conclusions

Changes in agglomerate structure were investigated by comparing values for D_f and A for 38 agglomerates sampled from ambient air to 39 from the concentrator exit. Figures 4 and 5 show the D_f distributions for concentrated and ambient aerosols, respectively. The count median D_f was very similar (between 1.6 and 1.8) for both concentrated and ambient particles. The average value of A for the particles collected from the VACES was 2.73 and for the atmospheric agglomerates 2.83.

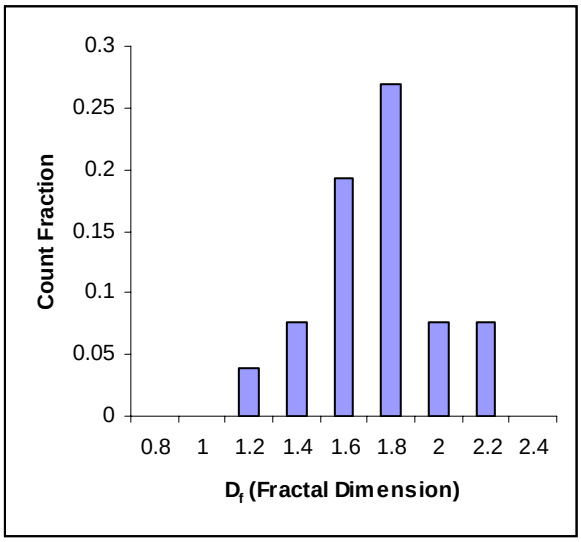

Figure 4. Fractal Dimension Distribution for Agglomerates From the VACES. The count mean D_f value was found to be between 1.8 and 2. Samples were taken at the Center for Health Sciences at UCLA on 8/22/00 using a LPI.

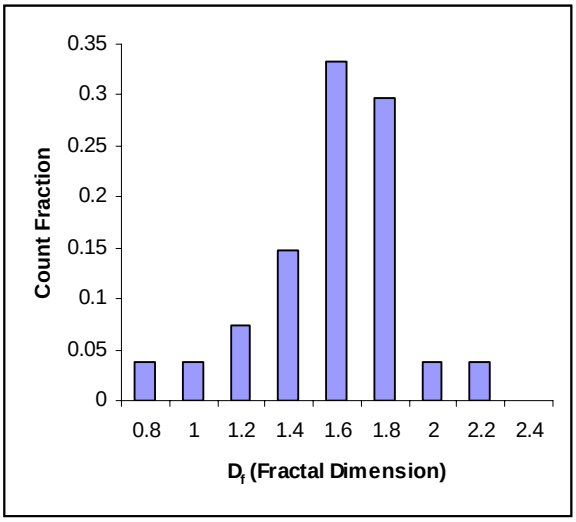

Figure 5. Fractal Dimension Distribution for Agglomerates Sampled From the Ambient Aerosol. The count mean D_f value was found to be between 1.6 and 1.8. Samples were taken at the Center for Health Sciences at UCLA on 8/22/00 using a LPI.

Previous studies suggest that chain agglomerates may become more compact when subjected to condensation and evaporation processes (Colbeck, et al., 1990; Hallet, et al., 1989; Wells, et al., 1976). In a study of diesel chain agglomerates (Huang, et al., 1994), the fractal dimension increased from 1.56 to 1.76 for mid-sulfur fuels and 1.40 to 1.54 for low-sulfur fuels, after condensation and evaporation processes. These were somewhat larger changes than we found. A possible explanation is that in the study by Huang, et al., the agglomerates underwent up to three cycles of condensation and evaporation while in our study they only went through one cycle. We therefore conclude that for the one set of measurements conducted by our Laboratory, the condensation and evaporation process used with the VACES did not cause significant changes in agglomerate structure as measured by D_f and A. However, both the sources of the fractal-like structures and associated trace gases may affect this phenomenon. Since the measurements were made for only one sampling site, more experiments will be needed at different sites to generalize these conclusions.

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Project 4: Effect of Exposure to Freeways with Heavy Diesel Traffic and Gasoline Traffic on Asthma Mouse Model (R827352C004)
Investigators: Michael Kleinman, Constantinos Sioutas, Arthur Cho, John Froines

Objective(s) of the Research Project: The objective of this study was to examine the effects of exposure to concentrated ambient particles (CAPs) from a heavily trafficked freeway on biomarkers of allergic and inflammatory responses in an asthma mouse model. A secondary objective was to examine pro-inflammatory biomarkers in brain tissue as a preliminary approach to the hypothesis that exposure to particulate matter could play a role in the onset or progression of neurodegenerative disorders, some of which can display an increase in oxidative and inflammatory events. Development of the particle concentrators and animal work were supported by funding from the California Air Resources Board as part of a five year program to develop and apply PM concentrators in health studies. The SCPCS supported concentrator operations, physicochemical characterization of the CAPs to which animals were exposed, and the examination of pro-inflammatory markers in brain tissue.

Summary of Findings: The goal of this study was to test the following hypotheses: (1) exposure to mobile emissions from mobile sources close to a heavily trafficked roadway will exacerbate airway inflammation and allergic airway responses in a sensitized mouse model, and (2) the magnitude of allergic airway disease responses will decrease with increasing distance

from the roadway. An ambient particle concentrator was used to expose ovalbumin (OVA)-treated BALB/c mice to purified air and to F and UF airborne particles at two distances, 50 m and 150 m, downwind of a roadway that is impacted by emissions from both heavy-duty diesel and light duty gasoline vehicles. Tissues and biological fluids from the mice were analyzed after exposures for 5 days per week in 2 consecutive weeks. The biomarkers of allergic or inflammatory responses that were assessed included cytokines released by Type 2 T-helper cells (IL-5, and IL-13), OVA-specific immunoglobulin E, OVA-specific immunoglobulin G1, and pulmonary infiltration of polymorphonuclear leukocytes and eosinophils. Allergy-related responses were increased in mice exposed to CAPs 50 m downwind of the road, compared to responses in mice exposed to purified air. No significant increases in allergy-related responses were observed in mice exposed to CAPs 150 m downwind of the road. The biological responses at the 50 m site were significantly associated with organic and elemental carbon components of F and UF suggesting that PM from motor vehicle fuel combustion could exert adjuvant effects and promote the development of allergic airway diseases.

We demonstrated that CAPs exposure increased inflammatory indices in brains of ovalbumin-sensitized BALB/c mice. Animals were divided into three exposure groups: filtered air (control), UF, or F and UF. The levels of proinflammatory cytokines interleukin-1 alpha (IL-1α) and tumor necrosis factor alpha (TNF-α) in the cytoplasm of brain tissue preparations were measured by immunoassay, and found to be increased in samples from mice exposed to particulate matter compared to that of control animals. Levels of the transcription factor NF-κB, which plays an important role in the expression of many immune system and pro-inflammatory compounds were also found to be substantially elevated in the brains of exposed mice compared with those of mice in the control group, as measured by an electrophoretic mobility shift assay. These findings suggest that components of inhaled particulate matter may trigger a proinflammatory response in nervous tissue that could contribute to the pathophysiology of neurodegenerative diseases. This small scale study is notable in that it identifies an important potential target tissue for the toxicity of particulate matter that has received little attention to date. If present in humans, brain inflammatory responses to particulate matter exposure could have a significant public health impact.

A full report to the CARB on this study is available for public download at:
http://www.arb.ca.gov/research/abstracts/98-316a.htm

Project 5: Effects of Exposure to Fine and Ultrafine Concentrated Ambient Particles near a Heavily Trafficked Freeway in Geriatric Rats (Pilot Project) (R827352C005)
Investigator: Michael Kleinman

Objective(s) of the Research Project: A geriatric rat model was used to test the hypothesis that free radicals produced by reactive organic and inorganic constituents of motor vehicle exhaust particles will induce injury to lung epithelium and changes in heart rate and blood pressure in aged animals exposed to UF and F near a freeway.

Summary of Findings: This study was carried out in two phases. In Phase 1, twelve rats aged 22 to 24 months were exposed 6 hours per day for two consecutive days to F and UF concentrated ambient particles (CAPs) at a site approximately 50 m downwind of a heavily

trafficked freeway. The average exposure concentration was about 300 μg/m^3. Control rats (n=10) were exposed to purified air. Four rats from the CAPs-exposure group were implanted with pressure transducers and transponders and their blood pressures and heart rates were measured. Measurements were made before and after each exposure. One of the implanted rats was euthanized because of a tumorous growth. Two weeks after the last CAPs exposure, the remaining implanted rats were exposed to purified air on 3 consecutive days, and blood pressure and heart rate were again measured before and after exposure.

Eight rats per group were euthanized 24 hours after their last exposure. Biochemical, cytological and histological assays were performed on blood, plasma, bronchoalveolar lavage, and heart and lung tissues. The endpoints included cell counts and cell differentials, inflammatory cytokines (TNFα, IL-1α, IL-1β, IFNγ) and anti-inflammatory cytokines (IL-4, IL-6, IL-10) in BAL. BAL from CAPs exposed animals had approximately 2 fold increases in some inflammatory and anti-inflammatory cytokines, relative to unexposed controls. IL-1α, IL-1β, IL-6 and IL-10 were increased in lung lavage fluid from exposed animals.

Heart rate and blood pressure were measured in the three surviving rats that had been implanted with cardiac monitors and telemetry transmitters. Average blood pressure and heart rate were both elevated when pre- and post-exposure measures were compared. The elevation in blood pressure after exposure was statistically significant, although the sample size is very small.

The surgical preparation of geriatric rats was difficult, and if scaled up, the protocol would benefit from implanting transmitters into rats at a younger age, before the exposure experiment was to commence. Notably, at a relatively low exposure concentration of F over just two days, we were able to detect post-exposure cardiovascular changes in three aged rats compared to controls and found some elevated cytokines in lavage fluid in a group of eight exposed animals.

A second exposure study was carried out in Phase 2. Thirty-four 22-month-old rats were exposed to filtered air (n=17) or $PM_{2.5}$ CAPs (n=17) at a site in Boyle Heights, CA with high levels of particulate pollution dominated by motor vehicle exhaust from freeway sources. Control group animals received filtered air. Blood pressure was obtained via tail cuff from all rats in the experiment. Electrocardiograph (ECG) parameters (heart rate [HR], heart rate variability [HRV]) and temperature were obtained from 5 animals in each group. Rats were monitored telemetrically for 20 minutes at the lab immediately before transport to the exposure site and twenty minutes at the lab immediately after the end of the exposure. Within 18–24 hours after the exposure ended, animals were euthanized by a lethal intraperitoneal injection of sodium pentobarbital (Nembutal, 65 mg/kg). Lung tissue samples were analyzed for malonaldehyde and glutathione as markers for oxidative stress, for IL-1β, TNFα, and IL-6, and for C-reactive protein. Western blots were analyzed for MAP kinases ERK1, ERK2, pERK2, JNK1, JNK2, p38 and pp38.

The concentration of CAPs ranged from 540–943 μg/m^3 (mean: 655 μg/m^3) at a site in Boyle Heights, CA with high levels of ambient particulate matter dominated by motor vehicle exhaust. With respect to cardiovascular responses, increased trends in the root mean square of successive differences in normal beat intervals (rMSSD) of HRV, a parameter highly correlated with vagal activity in the heart were observed on the second and third days of exposure. No significant

exposure-related changes in blood pressure or heart rate were observed. The cytokine concentrations were not significantly different between control and exposed rats. All of the MAPK's were lower in the exposed rats than in the control rats. The decreases were significant ($p \leq 0.05$) for ERK1 and pJNK. These MAPK's are involved in protection of cells from apoptosis and further study of these parameters is warranted in future studies.

Topic B: Studies of Emission Sources and Related Adverse Health Effects

Project 6: Ultrafine Particles and Freeways (R827352C006)
Investigators: William Hinds, Yifang Zhu, Constantinos Sioutas

Objective(s) of the Research Project:

Background

It is now well established that increases in the concentration of F in urban areas are associated with increases in morbidity and mortality. It is not known what properties of F cause these effects, but one candidate is UF. These are particles less than 100 nm or 0.1 μm in size and are found near combustion sources, such as motor vehicles. In an urban environment motor vehicle emissions usually constitute the most significant source of UF. However, there is very little information available either as to the size of the particles or the number concentration of UF emitted by vehicles in urban areas.

This research focuses on characterizing UF as they are transported away from a major emission source—a freeway. The overall objective of this research is to systematically evaluate and quantitatively predict UF particle concentration in the vicinity of freeways, particularly as they are transported downwind from freeways and into residences near freeways. Results from this study provide data and tools that allow epidemiologists and toxicologists to estimate exposure to UF in the vicinity of major highways.

Summary of Findings:

Particle Instrumentation Unit (PIU)

In collaboration with Dr. Constantinos Sioutas (USC), we designed, fabricated, and implemented a state-of-the-art movable PIU for research monitoring of the full size range of particulate air pollution at different locations in Southern California. The PIU is a movable laboratory designed to provide detailed characterization of the chemical and physical properties of ambient particulate matter, using state-of-the-art instrumentation, to support the SCPCS. The PIU is housed in a 20-foot long trailer, which was modified at UCLA and placed into service in Fall 2000. It has been in full operation at six locations in the LAB, Claremont, Downey, Riverside, Rubidoux, and USC for at least 3 months at each site. The PIU continuously measures the ambient particle size distribution over the particle size range of 10 nm to 20 μm as well as mass concentration and meteorological data, specifically, temperature, humidity, wind speed, and wind direction. Weekly particle size-segregated samples are taken with MOUDI cascade impactors for detailed chemical analysis. CO, ozone, and NO_x are monitored routinely.

Meteorological Tower

The lower part of the 10 m tower for the meteorological instruments consists of two 3-meter tower sections and the upper part is a 2-inch OD aluminum pole with a 1.25-foot fiberglass pole extension. The pole section is removable for transportation. With the base plate the tower weighs approximately 50 kg (110 lbs).

The tower is hinged at its attachment point at the top front of the trailer. The tower is well balanced and is easy and safe to lower and raise. It can be lowered or raised by one person for transportation in about 30 minutes. It can be partially lowered for calibration in 5–10 minutes.

Inlets

All sampling inlets that do not use a PM_{10} inlet were modified to use 180° stainless steel sweep elbows. This is to prevent rain from getting into the trailer or the sampling systems and instruments.

Pump Enclosures

Three noise reduction enclosures for sampling pumps were constructed and tested. Each unit consists of a lockable plywood box, 0.56 X 0.81 X 0.37 m high, on casters. Each unit can accommodate two large Gast vane pumps or three medium sized ones. The boxes are ventilated with an electric fan and all outlet air is HEPA filtered. The pumps can be turned on independently. A relay turns the cooling fan on if either pump is turned on. Semi-rigid fiberglass board lines part of the box for sound absorption. The enclosure reduces noise levels by 17.5 dBA.

Ultrafine Particles Near Freeways

To obtain data to model the concentration and size distribution of UF in the vicinity of freeways, we made detailed measurements in summer and winter of UF near the 405 and 710 freeways, two of the busiest in the country. Interstate 405 has more than 95% gasoline vehicles and Interstate 710 has up to 30% heavy-duty diesel vehicles; and thus these two roads represent the range of vehicle mix one is likely to encounter in urban areas.

Particle number concentration and size distribution in the size range from 7 nm to 220 nm were measured by a condensation particle counter (CPC) and a scanning mobility particle spectrometer (SMPS), respectively. Measurements were taken at 30 m, 60 m, 90 m, 150 m, and 300 m downwind and 300 m upwind from Interstate highway 405 at the VA National Cemetery and at similar positions near Interstate 710. At each sampling point, the concentration of carbon monoxide, black carbon and particle mass were also measured by a Dasibi CO monitor, an Aethalometer and a DataRam, respectively. Simultaneous measurements were made of wind speed and direction. Traffic volume was estimated by manually counting vehicles during replay of video recordings.

Results

A detailed description of the project's results can be found in Zhu, et al., 2002a, Zhu, et al., 2002b, and Zhu, et al., 2004. As shown in Figure 6, for the conditions of these measurements, the relative concentrations of CO, black carbon and particle number track each other well as one moves away from the freeway. Particle number concentration (6–220 nm) decreased exponentially with downwind distance from the freeway.

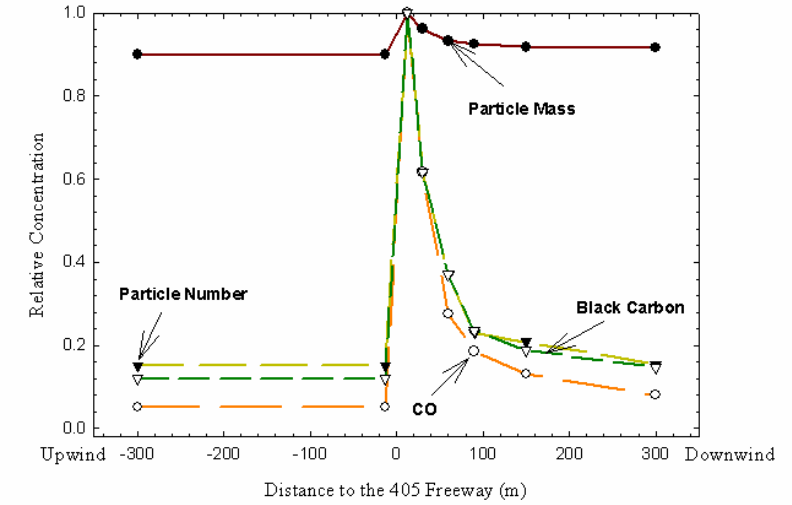


Figure 6. Relative Mass, Number, Black Carbon, CO Concentration Near the 405 Freeway

For the 405 freeway the average concentrations of CO, black carbon, particle number and PM mass at 30 m were in the range of 1.7 to 2.2 ppm, 3.4 to 10.0 μg/m^3, 1.3 × 10^5 to 2.0 × 10^5 /cm^3 and 30.2 to 64.6 μg/m^3, respectively. The total particle number concentration decreased exponentially with down wind distance from the freeway. The average traffic flow during the sampling period was 13,900 vehicles/hr. 93% of vehicles were gasoline powered cars or light trucks. The measured number concentration tracked traffic flow well. Thirty meters downwind from the freeway, three distinct modes were observed with geometric mean diameters of 12.6 nm, 27.3 nm and 65.3 nm, respectively. The smallest mode, with a peak concentration of 1.6×10^5 /cm^3, disappeared at distances greater than 90m from the freeway. Ultrafine particle concentration measured at 300 m downwind of the freeway was indistinguishable from upwind background concentration.

Atmospheric dispersion, coagulation, condensation and evaporation appear to contribute to the rapid decrease in particle number concentration and change in particle size distribution with increasing distance from the freeway. The maximum number concentration that was observed near the freeway was about 25 times greater than that for upwind locations. It suggests that people, who live, work, or travel on or within 100 m downwind of major traffic sources, will have much higher UF particle exposure than those who live farther away from such sources. The decay rates of CO and black carbon (BC) are slightly greater in summer than in winter for both freeways suggesting a weaker atmospheric dilution effect in winter. Particle number concentration in the size range of 6–12 nm is significantly higher in winter than in summer. The associated concentration in that size range decreased at a slower rate in winter than in summer. These results suggest that wintertime conditions favor greater UF particle formation, possibly due to increased condensation of organic vapors and slower atmospheric dilution.

A mathematical model was developed to predict UF particle number concentrations near freeways. Particle number based emission factors were estimated based on vertical measurement. Model inputs are traffic, wind, and location. Within the range of our data, the

model predicts particle number concentration near freeways with more than 90% accuracy. Atmospheric dispersion was found to be the dominant mechanism in determining the particle number concentration near freeways. With appropriate particle emission factors, traffic compositions, and meteorological data, particle number concentrations near freeways can be quantitatively estimated by this model (Zhu and Hinds, 2005). These data may be useful for epidemiological studies to estimate exposure to UF in the vicinity of major highways and to evaluate their adverse health effects. In collaboration with researchers from UC Davis, the dynamic changes of UF particle size distribution downwind of the freeways were also studied (Zhang, et al., 2004), and size-segregated emission factors were determined (Zhang, et al., 2005).

Similar measurements were repeated at night near the 405 freeway to study the diurnal pattern of UF near freeways. Average traffic flow at night was about 25% of that observed during the day. Particle number concentration measured at 30 m downwind from the freeway was 80% of earlier daytime measurements. This discrepancy between changes in traffic counts and particle number concentrations is apparently due to the decreased temperature, increased relative humidity, and lower wind speed at night. Particle size distributions do not change as dramatically as they did during the daytime. Particle number concentration decays exponentially downwind from the freeway similar to what was observed during the day, but at a slower rate. No particle number concentration gradient has been observed for the upwind side of the freeway. No $PM_{2.5}$ and very weak PM_{10} concentration gradients were observed downwind of the freeway at night. Ultrafine particle number concentration measured at 300 m downwind from the freeway was still distinguishably higher than upwind background concentration at night. Temperature and relative humidity affect UF particle formation significantly especially for the primary mode around 20 nm (Zhu, et al., 2006b).

Indoor and Outdoor (I/O) Relationships for Ultrafine Particles

Many urban residences, schools, and businesses are located in close proximity to high-density roadways. Consequently, indoor environments in urban areas may experience significant concentrations of outdoor UF. Given that people spend over 80% of their time indoors, understanding transport of UF from outdoor to indoor environments is important for assessing impacts of outdoor particulate matter on human health.

Indoor particle concentration is governed by indoor and outdoor sources, exchange rates, and particle physico-chemical characteristics. Indoor particle concentrations, therefore, depend on the dynamics of the transport and fate of outdoor particles in the indoor environments. Previous research in this area has focused on $PM_{2.5}$ and PM_{10} properties and behavior (Jones, et al., 2000; Thatcher and Layton, 1995). These studies found outdoor particles to be present at significant concentration in indoor spaces. In addition, the building shell was found to be ineffective in removing infiltrating particles. Considering health implications of UF particle exposure, it is important to assess particles' penetration characteristics into indoor environments and the relationship between their physical and chemical properties and infiltration. The overall objective of this project is to improve our knowledge of the indoor levels of UF from outdoor origin, especially those from motor vehicles in the vicinity of freeways.

Four two-bedroom apartments near the 405 Freeway in Los Angeles, CA were recruited for this study. Three of the four apartments (Apt 1, 2 and 3) are on the eastern side of the 405 Freeway. These three apartments are on the third floor with windows 3 m above a sound barrier wall next to the freeway. The horizontal distances between apartments 1–3 and the wall range from 15 m to 40 m. All three apartments are separated by no more than 50 m. The fourth apartment (Apt 4) is on the opposite, western, side of the 405 Freeway, 15 m from the sound barrier wall. Apt 4 is on the second floor with windows 0.5 m above the wall. All the apartments are about 8 years old with central mechanical ventilation systems that can be turned off. Dominant wind direction during the day is from west to east (sea breeze) and at night times winds are light with a slight east to west flow.

Indoor and outdoor UF particle size distributions (6 nm to 220 nm) were measured concurrently under different ventilation conditions without indoor sources or aerosol generation activities. Figure 7 shows averaged particle size distributions and indoor/outdoor ratios for apartment 1. Figure 7a shows day time (10 am–5 pm) and Figure 7b shows night time indoor and outdoor particle size distributions. Figure 7c shows particle size dependent indoor/outdoor ratios during day and night times.

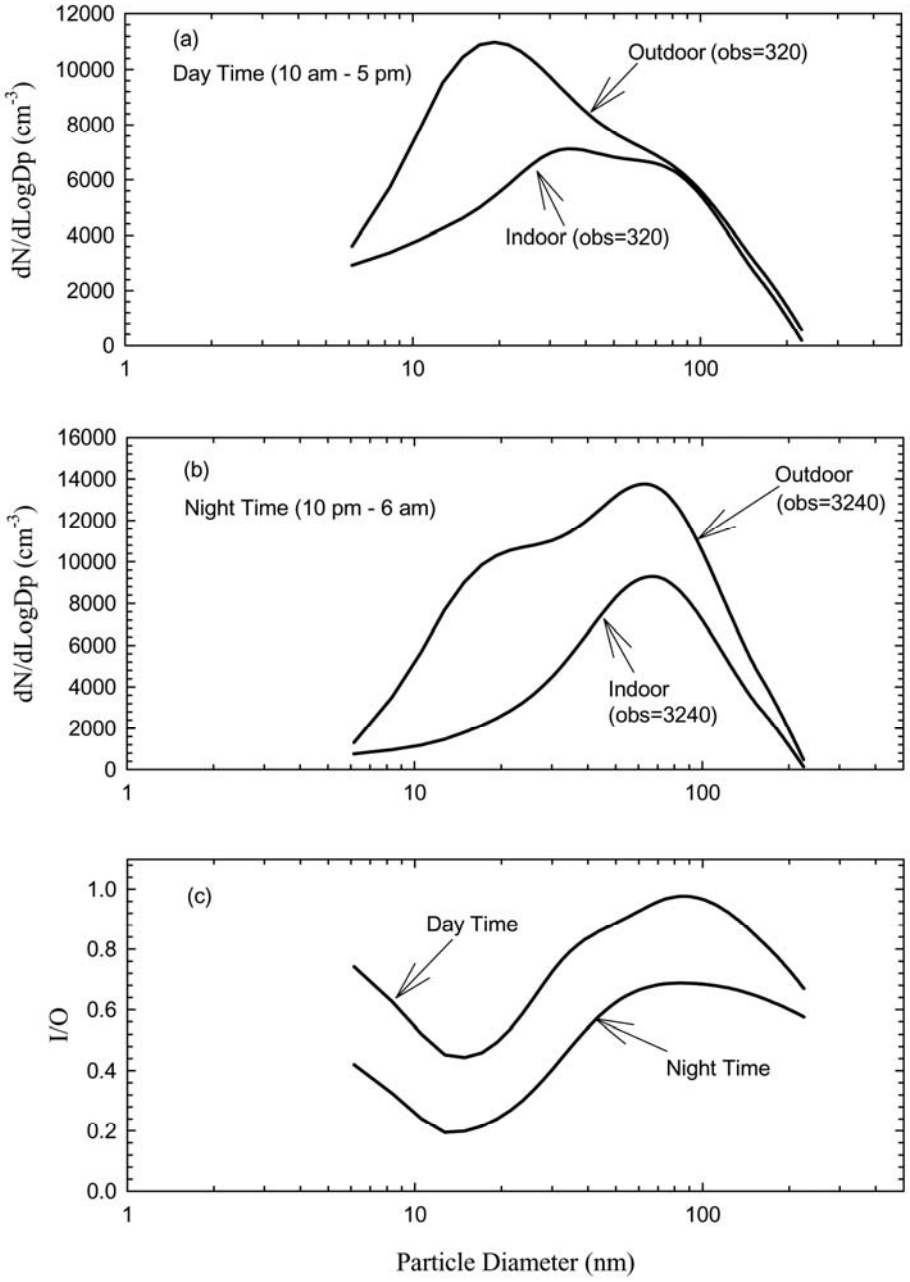
Figure 7(a) shows a daytime outdoor particle size mode near 20 nm, consistent with previous reports (Zhu, et al., 2002b). No such mode exists for indoor observations, and indoor particle number concentration is much more stable than outdoors. Night time particle number concentrations, shown in Figure 7(b), are comparable to their day time values. Although traffic densities are lower during the night, vehicle speeds on the freeway are much faster. It has been shown previously that faster vehicles generate more particles (Zhu, et al., 2002b). Lower nighttime temperatures, may also result in higher emission factors for particle number, as described by Kittelson (1998) and observed in the nighttime study (Zhu, et al., 2006b). Another reason for higher particle number concentrations during the night may be lower wind speeds, and a lower atmospheric mixing height, thus weaker atmospheric dilution effects.

As Figure 7(c) shows, I/O ratios during day and night times exhibit similar trends and shapes. Day and night I/O profiles for particles above 20 nm are consistent with theoretical curve shapes. Curves for particles below 20 nm do not correspond to the accepted theory, as no downward trend is observed for both day and night time observations. One possible reason is the low instrument detection limit in that size range, and thus large variability and less statistical confidence in data on particles below 20 nm. Another possible reason may be the unique, semi-volatile, nature of freeway UF. Freshly emitted freeway UF are known to have a considerable fraction of volatile components, especially particles below 50 nm (Kittelson, 1998). For example, some of the particles in the 20–40 nm size range may lose their volatile components and become particles of 20 nm or less. Such loss of volatile components has been observed previously (Lunden, et al., 2003). The volatility properties of freeway UF were reported in a companion paper to this study (Kuhn, et al., 2005). The difference between day and night I/O may be due to higher air exchange rates during daytime.

Pilot Study of I/O Relationships for Vehicles

Very limited information is available on human exposure to freshly emitted UF while commuting on major roads and freeways. We conducted experiments to measure in-cabin and outdoor particle number concentration and size-distributions while driving three vehicles on Los Angeles freeways. Particle number concentrations and size distributions were measured under different operating conditions of vehicle ventilation system (windows open, AC on/off, recirculation on/off). Outside changes in particle counts caused corresponding in-cabin changes approximately 30–40 s later, indicating an air exchange rate of about 100 hr^{-1} when the fan and air conditioning were set to on. Maximum in-cabin protection (~85%) was obtained with ventilation conditions of "recirculation on" and high fan speeds. In-cabin and outdoor particle size distributions in the 7–300 nm range were observed to be mostly bimodal, with the primary peak occurring at 10–30 nm and the secondary peak occurring at 60–100 nm. The factory-installed particle filter in the vehicle ventilation system offered an in-cabin protection of about 50% for particles in the 7–40 nm size range, and 20–30% for particles in the 40 to ~200 nm size range. Based on these results, a manuscript has been submitted for publication (Zhu, et al., 2006a).

Figure 7. Averaged (a) Day Time, (b) Night Time Outdoor and Indoor Particle Size Distributions and (c) Size Dependant I/O Ratios in Apt 1.



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Project 7: Exposure to Vehicular Pollutants and Respiratory Health (R827352C007)
Investigators: Robert McConnell, Fred Lurmann, W. James Gauderman, Edward Avol

Objective(s) of the Research Project:

Project Hypotheses

This project tested the hypothesis that exposure to vehicular pollutants is associated with respiratory health in children. We have examined this hypothesis using data from the Children's Health Study (CHS), a longitudinal evaluation originally designed to evaluate the effect of average community pollutant exposures on average community respiratory health outcomes. Pollution has been well characterized at central site monitors in 16 southern California communities involving participants from two sets of school based cohorts. In the SCPCS we have characterized within-community variation in traffic related pollutant exposure. These new pollutant metrics have allowed us to examine the relationship of individual level exposures to the CHS outcomes:

1. We have developed modeled metrics of exposure and tested these against measured pollutants.

2. We have evaluated the association of these metrics and of measured indicator pollutants to:

    a. Prevalence and severity of asthma at entry
    b. Incidence of asthma during follow-up
    c. Lung function at study entry and its growth during follow-up
    d. School absence.

3. We have evaluated the association of chronic asthma exacerbation with chronic temporal variation from yearly variation in exposure to oxidant pollutants among asthmatics.

Project Objectives

Our approach has been to geo-code addresses of residences and schools and then to assign traffic exposures to these addresses, based on traffic estimates available from the California Department of Transportation (Caltrans). We have used ambient air quality measurements at 12 locations to evaluate these models. Using modifications of statistical modeling strategies developed for the CHS, we have examined the effect of exposure to traffic related pollutants on asthma prevalence, and we have found relationships not previously reported in this growing literature. In addition, we have examined outcomes for which there has been little previous study of the effects of traffic modeled pollutants: asthma severity, asthma incidence, lung function and lung function growth, and school absence.

Summary of Findings:

Aim 1: Characterization of Exposure to Traffic-Related Pollutants in CHS Communities

The overall objective of the exposure component of this study was to provide improved methods and databases to characterize seasonal and annual average population exposure to traffic-related pollutants with fine spatial resolution. Prior to this study, Sonoma Technology, Inc. had performed some initial traffic modeling for the CHS which pointed to the need for model refinements, database improvements, and model performance evaluation. Many potential improvements were explored; improvements in the spatial accuracy of roadway and receptor data, meteorological data, and emission rates proved most important. It is important to note that most assessments of traffic-related pollutant exposure focus on worst-case 1-hr or 8-hr maximum conditions. Our focus was on estimation of long-term exposures in order to evaluate relationships with chronic health effects.

Traffic Activity and Roadway Data. Annual average daily traffic counts data for Interstate freeways, other principal arterials, minor arterials, major collectors, and minor collectors in 2000 were obtained from Caltrans. The annual traffic counts are based on continuous measurement data for freeways and intermittent measurements (usually every three years) on other arterials and some collectors. Diurnal traffic volume variations and day-of-week variations for light-duty and heavy-duty vehicles on freeways were determined from Caltrans weigh-in-motion (WIM) data for Southern California. Diurnal variations for collectors were determined from more limited traffic measurements (Chinkin, et al., 2003).

ESRI ArcGIS software was used to process the Caltrans roadway link and traffic count data. The Caltrans roadway geometries were mostly based on TIGER files and were often inaccurate, based on comparisons with aerial photography images. Comparison to global positioning system (GPS)-accurate TeleAtlas Roadway Network data showed that the Caltrans' TIGER roadway links occasionally had 250-m discrepancies from actual roadway locations. The roadway geometry errors were random and affected roadways of all sizes in most communities. Zhu, et al. (2002) reported ten-fold differences in measured concentrations of traffic-related pollutants between 30 m and 200 m downwind of Southern California freeways. Errors of 100 m to 250 m in the location of major roadways relative to residence are not acceptable for neighborhood-scale assessments of traffic effects. Since the Caltrans roadway location data did not have sufficient accuracy for our intended use, methods (software) were developed to transfer the Caltrans annual traffic volumes to the GPS-accurate TeleAtlas roadway network. The TeleAtlas roadway database incorporated both more accurate and more precise location information. For example, each direction of travel on moderate and large roadways is represented as a separate link in the TeleAtlas database.

Proximity Modeling. We wanted to examine the relationships of CHS participant health status with traffic indicators separately from the CALINE4 dispersion model estimates of concentrations from mobile source emissions. Dispersion modeling provides refinements but introduces additional uncertainties compared to analysis of traffic alone. A three-level hierarchical approach was adopted for traffic assessment that considered: (1) the distance of residences to nearest roadways of various types, (2) GIS-mapped traffic density assignments at residences, and (3) the CALINE4 dispersions model estimates of traffic-related pollutant concentrations at residences.

The first sets of traffic metrics were the distances from residences to the nearest roadways of different types. GIS tools were used to calculate the distance to the nearest: (1) interstate freeway,

U.S. highway, or limited access highway; (2) other highways; (3) arterial roads; (4) collector roads; and (5) local roads.

The second approach for characterization of traffic exposures was to calculate traffic densities, which vary more smoothly in space than the distances to nearest roads. They also capture the effects of intersection and multiple roadway influences that are missed using only distance to the nearest roadways. The link-based traffic volumes are used to generate maps of traffic density using the ArcGIS Spatial Analyst software. The traffic density maps were created with a Gaussian decay function that has traffic densities decreasing by ~90% between the roadway and 150 m away (perpendicular) from the roadways, which is consistent with the characteristics observed by Zhu, et al. (2002).

Dispersion Modeling. The CALINE4 model, developed by Caltrans and the U.S. Federal Highway Administration (Benson 1989), is one of several Gaussian line source dispersion models that is designed to estimate local-scale pollutant concentrations from motor vehicle emissions. It has primarily been evaluated for inert traffic-related pollutants such as CO over short periods and was selected for the SCPCS analyses because it has a credible scientific formulation. The model was used to simulate ambient concentrations due to on-road motor vehicle emissions on all roads with traffic volume data located within a 20 km square centered in each CHS community.

A climatological approach was used to estimate long-term average concentrations. The model was applied for a wide range of meteorological cases in each community, and the seasonal or annual concentrations were calculated by weighting the results for individual cases by the frequency of occurrence of the conditions in the community. The results are post-processed to incorporate pollutant-specific emission factors, diurnal and day-of-week variations of traffic volumes, and chemical conversion (for NO to NO_2).

Vehicle emission factors were obtained from the CARB's EMFAC2002 vehicle emissions model. The elemental carbon (EC) and organic carbon (OC) fractions of exhaust PM emissions were based on composite profiles from Gillies and Gertler (2000). Paved road-dust emission factors for $PM_{2.5}$ and PM_{10} were based on Southern California in-roadway measurements (Fitz and Bufalino, 2002). The EMFAC model also estimates the PM emissions from brake wear and tire debris.

Figure 8 shows comparisons of CALINE4 model estimates for the air monitoring station locations to the 4-year average observed ambient concentrations at the stations. There were correlations between the model estimates and the observations for all pollutants. The dispersion model estimates local motor vehicle emissions contributed 17% of the observed NO_2 concentrations, on average, and the coefficient of determination was 0.81. The model estimates for NO_x were more strongly correlated with observations ($r^2 = 0.97$) than those for NO_2. This result was consistent with the dispersion model being formulated for chemically non-reactive species and, therefore, the model was more accurate for NO_x than NO_2. The average estimated contribution of local motor vehicle emissions to the observed $PM_{2.5}$ EC concentrations was 16% and the coefficient of determination was 0.86. The relationships of the model results to the observed concentrations were very similar for $PM_{2.5}$ EC and NO_2. This was somewhat surprising given that EC emissions from vehicles are not well characterized and most emissions experts consider PM (and EC) emissions rates to be far more uncertain than those for NO_x. The model estimates for $PM_{2.5}$ OC were less well correlated with the

observed concentrations ($r^2 = 0.71$). The model estimate for the Mira Loma station location was quite low compared to the very high OC concentration observed in the station. The comparison of estimated and observed $PM_{2.5}$ OC was confounded by not only the contributions of regional transport and other local sources, but also the contribution of secondary OC to the measured OC. The various estimates of the incremental traffic related pollutant contributions to the background concentrations were, in general, highly correlated. NO_2 and NO_x have been used in the CHS and other population based studies as indicators of within-community variation in exposure to traffic-related pollutants that can be measured at a reasonable price. For consistency we have examined exposure to these pollutants, but the associated health effects of modeled exposure from traffic might plausibly be attributed to particulate exposure or to other gaseous co-pollutants in tailpipe emissions.

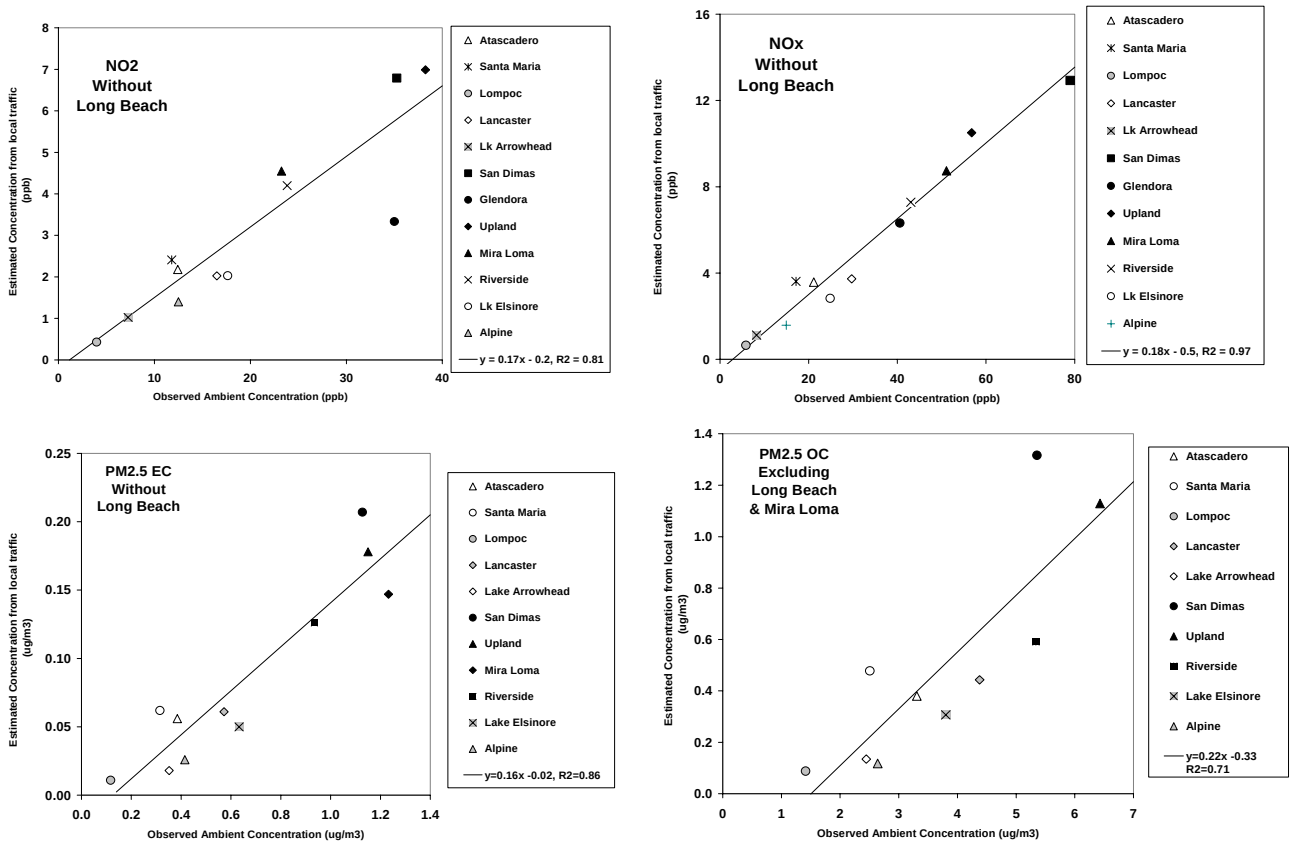

Figure 8. Comparison of Annual Average NO_2, NO_x, EC, and OC Concentrations Estimated by the CALINE4 Model for the Central Air Monitoring Station Locations and the Four-Year Average Observed Ambient Concentrations at the Stations. Note: the observations for San Dimas are based on only two years of data.

Simulations of concentrations from local roads in CHS communities confirmed the importance of regional-scale and urban-scale pollutant sources and meteorological transport in southern California. At the types of locations selected for community monitoring, the ambient pollutant concentrations due to transport from upwind and local non-mobile sources were always larger than the simulated concentrations from local on-road motor vehicle sources. This finding was consistent with regional modeling results completed for another SCPCS project led by Dr. Arthur Winer.

The CALINE4 dispersion model results were also used in the modeling of personal exposure of CHS participants. They were used as input to the Individual Exposure Model (IEM) developed under the SCPCS project led by Dr. Arthur Winer and described in Wu, et al., 2005b.

We have also examined the relationship between traffic modeled pollution and variation in ozone within communities (McConnell, et al. 2006b). In high ozone communities, ozone concentrations might be expected to vary inversely with fresh traffic exhaust, because NO in fresh exhaust scavenges ozone. To explore this hypothesis, we used a sample of homes for which ozone was measured simultaneously at the home and the central site monitor, as part of a previous study (Avol, et al. 1998). There was a highly significant inverse relationship observed between measured ozone and our new traffic modeled exposure to oxides of nitrogen that varied spatially within communities. Although it is well known that ozone concentrations are low near major roadways, there has been little previous systematic study of the pattern of spatial variation within communities due to scavenging of ozone. Because ozone and traffic related particulate are both toxic to the lungs, and they are inversely correlated with respect to traffic, the risk of traffic exposure might be expected to be attenuated in high ozone communities. With separate funding we have embarked on a new study to measure ozone and NO_x at homes of children with and without incident asthma to explore the significance of these traffic-ozone relationships.

Aim 2a: Effect of Traffic on Asthma Prevalence and Severity

We evaluated the risk of lifetime asthma at study entry and traffic related pollutant exposure. We observed large risks associated with measured NO_2 at a sample of homes of children recruited into the original CHS study in 1993 and 1996 (Gauderman, et al., 2005). There were also high risks associated with residential distance to a freeway and with freeway modeled exposure to traffic related pollutants, using modeled exposures developed in Aim 1. The strength of this study is that we measured a traffic related pollutant at the homes, which has not been the case for most studies of traffic and asthma, and the consistency of results from measured and modeled exposures makes a causal relationship more plausible. Using modeled exposures, we observed a similar association in the entire cohort (unpublished data).

We then examined risks associated with early life asthma in younger children (5–6 years old) in a new cohort of CHS children recruited in 2003, using modeled exposures. The pattern of results largely replicated those observed in the earlier cohorts, and we found a strong relationship between prevalent and lifetime asthma and modeled exposure. In addition, there was a strong relationship with a relatively simple traffic metric, distance to a major road (McConnell, et al., 2006a) (see Table 4).

Table 4. Association of Asthma and Wheeze With Traffic Related Pollution Among Long Term Residents

		Lifetime Asthma		Prevalent Asthma		Current Wheeze	
	N*	O.R.*	(95% C.I.)	O.R.*	(95% C.I.)	O.R.*	(95% C.I.)
Major road distance							
>300 m	813	1.00		1.00		1.00	
150 - 300 m	483	0.86	(0.59 , 1.24)	0.83	(0.56 , 1.21)	0.97	(0.69 , 1.38)
75-150 m	294	1.03	(0.68 , 1.56)	1.09	(0.71 , 1.66)	1.09	(0.73 , 1.62)
<75 m	266	1.46	(0.98 , 2.17)	1.64	(1.10 , 2.44) †	1.67	(1.14 , 2.43) §

*N is total exposed in each category of exposure; O.R. (95% C.I.) odds ratio (95% confidence interval), adjusted for age, sex, language of questionnaire, community, and race.
†p<.05; §p<.01

The effect varied by parental history of asthma and age of exposure, with much stronger associations observed in those with exposure before age 3 and with no family history. We believe this is an important finding, as some of the inconsistency in the literature may be explainable based on different proportions of susceptible children in different studies. Among children with a lifetime history of asthma in this cohort, we have also examined the relationship of traffic-related pollutants to various indicators of asthma severity, including wheeze and bronchitic symptoms. Traffic related pollution was associated with bronchitic symptoms (unpublished results). This outcome is an indicator of chronic asthma exacerbation, which we have previously shown to be a sensitive endpoint for particulate effects (McConnell, et al., 1999).

During the past year we have undertaken a pilot study to evaluate the relationship of traffic modeled pollutants to measured particulate and NO_x pollution in Long Beach, a CHS community with heavy primary particulate pollution. We used nephelometers as indicators of $PM_{2.5}$ pollution that could be measured at reasonable cost in a relatively large number of sites simultaneously. NO, NO_2, and ozone were measured using integrated passive samplers. Residences of cases of lifetime asthma and controls from the 2003 cohort formed the sampling frame. The goal was to see if short term sampling at many locations would accurately indicate long term intra-community variation in exposure (and predict health effects), if the short term samples were co-located with a central site monitor for which continuous historical data are available. The nephelometers were co-located with the Ogawa ozone and NO_x samplers during 7 two week cycles at a total of 69 locations. There were 8 to 10 different residences per cycle. In addition, measurements were made at a central site and 4 other locations during all sampling periods. Traffic related exposure at all locations also was modeled. At selected sites, filters were also collected for PM_{10}, $PM_{2.5}$, and $PM_{0.25}$ EC and OC. Preliminary results show that measurements made with nephelometers were only weakly associated with NO, NO_2 and ozone. This indicates that NO_2, which is often measured as a cheap surrogate for traffic related pollutants, is not a good indicator of intra-community variation in $PM_{2.5}$. Traffic modeled exposure was associated with lifetime asthma, but measured pollutants were not. Likely explanations for this discrepancy include the short sampling period, which was unlikely to reflect annual or lifetime exposure. As expected, the measurements made at the community central site monitor varied by sampling period (due to varying meteorology). However, neither the residual of measurements at the central site and co-located locations in every cycle, nor the ratio of co-located measurements to the central site, were constant across cycles. A clear lesson for future epidemiologic studies is that short term sampling may not be adequate to characterize long term exposures, even if co-located with central site monitoring for which longer term exposure is well

characterized. A manuscript is in preparation. These data have also been used for complementary analyses in SCPCS supported work conducted by Krudysz and Sioutas.

Aim 2b: Effect of Traffic on Incident Asthma

Although there is a growing literature on studies of prevalent asthma and traffic, few previous studies have examined the relationship of traffic related pollutants with incident asthma. We have examined the effect of traffic modeled exposures on incident asthma in both sets of CHS cohorts. In the older cohorts there was an association of incident asthma with traffic related pollutants at the sample of homes at which NO_2 was measured (Jerrett, et al., 2006). The effects of modeled exposure in the entire cohort were weaker and sensitive to the modeling strategy. Analyses of those data are ongoing. Among children recruited at age 5–7, early follow-up indicates that CALINE-modeled exposure is associated with new onset asthma. Results are stronger among children who had no history of wheeze at study entry (and who were therefore less likely to have pre-existing asthma that was merely exacerbated by exposure). The effects of traffic related exposure are independent of cross community effects of ozone that also were observed and which are compatible with the previous associations of incident asthma with long term average ozone exposure we have observed in the CHS (McConnell, et al., 2002). A manuscript is in preparation.

Aim 2c: Effect of Traffic on Lung Function and Lung Function Growth

We have shown deficits in community average lung function in the CHS and particulate measurements made at central site monitors. Deficits in flow rates were observed at study entry and in replicated assessments of 4-year lung function growth rates in different cohorts of children (Gauderman, et al., 2002; Gauderman, et al., 2000; Peters, et al., 1999). Recently, we reported deficits in 8-year growth rates (Gauderman, et al., 2004). These findings are part of an emerging literature indicating that traffic related pollutants compromise lung function. However, there has been little study of within-community variability in traffic-related pollution and lung function and no previous study of its association with childhood lung function growth. We have examined the association of lung function deficits at study entry using a novel Bayesian estimator of NO_2 exposure at homes, using both measured and traffic modeled indicators of exposure (Molitor, et al., 2006). We found deficits in both lung flows and lung volumes associated with exposure to NO_2, suggesting a detrimental effect of traffic related pollutants.

We have now incorporated traffic modeled exposure into the evaluation of 8-year lung function growth in the CHS. Effects of traffic modeled exposure were independent of and similar in magnitude to the effects of previously reported regional background particulate pollution (Gauderman, et al., 2004) measured at the central site monitors. Thus, both local traffic and ambient background $PM_{2.5}$ and PM_{10} were associated with impaired lung function. These results are important because impaired lung function in childhood is associated with lung deficits in adulthood. In adults, impaired lung function is a strong predictor of respiratory morbidity and death. A manuscript is in review (Gauderman, et al., 2006).

Aim 2d: School Absence and Traffic

School absences are an outcome not initially proposed for this study, but we have observed strong associations with CALINE4 derived traffic metrics and distance to a freeway. The effects were observed exclusively among children with asthma. A manuscript is in preparation.

Aim 3: Chronic Asthma Exacerbation is Associated With Yearly Variation in Central Site Particulate Measurements

Most of our analyses have examined the effect of spatial variation within communities in pollutants modeled from traffic or measured at a sample of homes. Many other studies have examined acute effects of temporal variation in pollution over days to weeks, but little previous work has examined the effect of temporal variation over longer periods on chronic respiratory outcomes.

We developed novel statistical methods to evaluate the effect of year-to-year temporal variation in the average ambient pollutants measured at the central monitoring sites in each community. The relationship of bronchitic symptoms to ambient particulate matter and to particulate elemental and organic carbon (OC), nitrogen dioxide (NO_2) and other gaseous pollutants was examined in a cohort of the asthmatic children in the CHS (McConnell, et al., 2003a). Symptoms, assessed yearly by questionnaire from 1996–1999, were associated with the yearly variability of particulate matter with aerodynamic diameter less than 2.5 μm (odds ratio [O.R.] 1.09/$\mu g/m^3$; 95% confidence interval [C.I.] 1.01–1.17), OC (O.R. 1.41/$\mu g/m^3$; 95% C.I. 1.12–1.78), NO_2 (O.R. 1.07/part per billion (ppb); 95% C.I. 1.02–1.13) and ozone (O.R. 1.06/ppb; 95% C.I. 1.00–1.12). The odds ratios associated with yearly within-community variability in air pollution were larger than the effect of the between-community four-year average concentrations. In two pollutant models, the effects of yearly variation in OC and NO_2 were only modestly reduced by adjusting for other pollutants, the effects of all other pollutants were reduced after adjusting for OC or NO_2. We concluded that previous cross-sectional studies of bronchitic symptoms may have underestimated the risks associated with air pollution. In subsequent analyses, we have observed that the yearly variation in the effect of air pollution in these asthmatic children occurred exclusively among children with a dog (but not a cat) in the home (McConnell, et al., 2003b). We hypothesized that this interaction of pollution with dog ownership could be an indication of up-regulation of asthmatic response to air pollution by exposure to endotoxin, which has been shown in other studies to be present in higher concentration in homes with a dog. A manuscript is under review.

Conclusions

1. Proximity to high traffic corridors and exposure to intra-community variability in traffic-related pollutants was associated with lifetime asthma in cross sectional analyses and with incident asthma during follow-up of the CHS cohorts.

2. Lung function and lung function growth were associated with traffic related air pollution estimated both at the home and at the central site monitor.

3. School absence was associated with traffic related air pollution at home and school among children with asthma.

4. Yearly variation in organic carbon and other particulate pollutants were strongly associated with bronchitis among children with asthma; these effects were modified by a dog in the home, an indicator of endotoxin exposure.

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Project 8: Traffic Density and Human Reproductive Health (R827352C008)
Investigator: Beate Ritz

Objective(s) of the Research Project: When we examined associations between adverse birth outcomes and maternal air pollution exposure during pregnancy based on a cohort of Southern Californian births during 1989–1993, we found that increases in carbon monoxide (CO), particulate matter less than 10 microns (<10 μm) in aerodynamic diameter (PM_{10}), and ozone (O_3) concentrations during vulnerable pregnancy periods increased the risk of term low birth weight (LBW) (Ritz and Yu, 1999), preterm delivery (Ritz, et al., 2000), and common cardiac malformations, such as ventricular septal defects (Ritz, et al., 2002). Carbon monoxide is released directly in motor vehicle exhaust and does not react readily in the atmosphere to form other compounds. The consistently observed associations between ambient CO and adverse birth outcomes in our studies suggest that compounds in motor vehicle exhaust may affect fetal development. CO, however, may be only an easily measured and stable indicator for other compounds emitted in exhaust. For example, F and UF are also released directly in vehicle exhaust, and UF number count and CO concentrations correlated almost perfectly when measured with increasing distance from a roadway out to 300 feet (Zhu, et al., 2002a,b). When we used a census-based measure of commuting level, we also found higher risk of term LBW for women who commuted more than 60 minutes to work (Ritz and Yu, 1999). These findings, along with data indicating pollutant concentrations inside vehicles are generally higher than ambient concentrations (e.g., Rodes et al., 1998 and Fruin, 2003), suggest in-vehicle exposure to motor vehicle exhaust pollutants while commuting may also be an important risk factor for adverse birth outcomes in pregnant women.

Therefore, the first research goal of this project was to determine whether residential proximity to heavy traffic roadways, such as freeways and major arterials, affected the risk of LBW and preterm birth in infants born to women living in Los Angeles County, California between 1994–2000. Residential proximity to heavy traffic roadways was used as a surrogate measure of exposure to motor vehicle exhaust. The second research goal of this project was to evaluate whether maternal in-vehicle air pollutant exposures during commutes affected the risk of LBW and preterm birth in infants born to women living in Los Angeles County, California during 2003, who responded to a survey within 6 months of delivery (the EPOS study survey).

Summary of Findings:

Achievement of Project's Objectives/Accomplishments

First, we used an epidemiologic case-control study design to examine whether residential proximity to heavy-traffic roadways influenced the occurrence of LBW and/or preterm birth in Los Angeles County between 1994 and 1996 (Wilhelm and Ritz, 2003). We mapped subject home locations at birth and estimated exposure to traffic-related air pollution using a distance-weighted traffic density (DWTD) measure. This measure takes into account residential proximity to and level of traffic on roadways surrounding homes. We calculated odds ratios (ORs) and risk ratios (RRs) for being LBW and/or preterm per quintile of DWTD. The clearest exposure-response pattern was observed for preterm birth, with a RR of 1.08 (95% confidence interval [CI]=1.01–1.15) for infants in the highest DWTD quintile. Although higher risks were observed for LBW infants, exposure-response relations were less consistent. Examining the influence of season, we found elevated risks primarily for women whose third trimester fell during fall/winter months ($OR_{term\ LBW}$= 1.39; 95% CI=1.16–1.67; $OR_{preterm\ and\ LBW}$ = 1.24; 95%

CI=1.03–1.48; $RR_{all\ preterm}$ = 1.15; 95% CI=1.05–1.26) and exposure-response relations were stronger for all outcomes for these women. This result is consistent with elevated pollution in proximity to sources during more stagnant air conditions present in winter months.

In a second study (Wilhelm and Ritz, 2004), we expanded our analysis to the time period 1994–2000 and incorporated available information on the number of trucks frequenting freeways in our study area. The number of trucks frequenting a given freeway may be important since some gasoline-fueled trucks emit more CO than passenger cars (on a per vehicle basis) and diesel-fueled trucks emit greater quantities of UF than passenger vehicles (Zhu, et al., 2002a,b). We mapped subject home locations at birth and estimated DWTD and the number of trucks on freeways within 750 feet of each residence. Although Caltrans provides annual average daily traffic (AADT) values for roadway segments in LA County, these data do not differentiate between gasoline and diesel-fueled vehicles on regular streets. However, this agency does collect data on the number of trucks at various freeway locations throughout the state, listing the annual average number and percentages of 2-, 3-, 4- and 5 or more axle trucks passing a given count location within a 24-hour period. Since a truck traffic count is not available for every freeway segment, the count data were extrapolated between count locations based on the postmile (i.e., location) and leg (i.e., direction) information provided in the truck count data. We used these data to determine the total number of trucks on freeways within 750 feet of subject residences. We also estimated the number of heavy-duty diesel trucks assuming 43% of the 2-axle (6 tire), 90% of the 3-axle and 100% of the 4-axle and 5 or more axle trucks were heavy-duty diesel. These percentages are based on truck census data reported by Dreher and Harley (1998). Odds ratios (ORs) for term LBW and preterm and LBW (preterm-LBW) birth, and risk ratios (RRs) for preterm birth were estimated based on quintiles of the DWTD distribution and the 90th and 95th percentiles of the freeway truck distributions using logistic regression.

We did not observe associations between DWTD and LBW (term and preterm) for 1997–2000, but in certain subgroups we still observed associations with preterm birth: women whose third trimesters fell primarily during fall/winter months (November–April) (RR=1.07, 95% confidence interval [CI] = 0.99–1.16, comparing the highest to lowest DWTD quintile) and women living in census block groups with a fraction of children in poverty at or above the median value (RR=1.08; 95% CI=1.00-1.18). However, we observed a 23% greater risk of preterm-LBW birth for women with ≥13,290 freeway trucks passing within 750 feet of their residence per day (95th percentile) (OR=1.23, 95% CI=1.06–1.43) during 1997–2000.

The limitations associated with using DWTD as a measure of motor vehicle exhaust exposure in pregnant women is discussed in Wilhelm and Ritz (2003). Previously we noted that the DWTD model assumes motor vehicle exhaust dispersion follows a Gaussian curve centered on a given roadway with 96% decay occurring at 500 feet (152.4 meters) and that such a curve may not adequately represent dispersion conditions since meteorologic factors such as wind direction, wind speed and inversion layer height may be important. We tried to address this issue in models that incorporated some simple adjustments for wind direction and wind speed. We accounted for the percentage of time each residence was downwind of a given street during certain pregnancy periods of interest, i.e., each home and street-specific DWTD value was weighted by one during hours when the home was downwind of the street and by zero during hours when the home was not downwind of the street. Residences were considered downwind of

a street during hours when the wind direction was perpendicular to the street (in the downwind direction) ± 45 degrees. The pregnancy periods of interest were the third trimester for term LBW and six weeks prior to birth for preterm birth. These periods were selected based on our previous findings that showed the greatest associations between background air pollution and these adverse birth outcomes during these periods (Ritz and Yu, 1999 and Ritz, et al., 2000). After each home and street specific DWTD value was multiplied by the percentage of time downwind, these values were summed for all streets within the 750-ft buffer to obtain the final wind-direction adjusted DWTD value for each subject. In addition to incorporating wind direction into the DWTD measure, we also incorporated wind speed. Each home and street-specific DWTD value was multiplied by the percentage of time downwind and by 1/average wind speed during these downwind periods, i.e., we assumed a simple inverse relationship between concentrations of motor vehicle exhaust pollutants within 750 feet of the roadway and wind speed. Measurements of UF and CO near roadways indicate this assumption is reasonable for a simple modeling approach (Zhu, et al., 2002a,b; Benson, 1984). In general, these modifications of our original DWTD estimates did not change or improve the exposure-response patterns between DWTD and the outcomes.

In the work summarized above, we used electronic birth certificate data as the source of information on both health outcomes and other covariates we evaluated in our statistical models. However, the birth certificate data are missing information on some potentially important risk factors such as smoking, maternal stature and weight gain during pregnancy, and stress. We therefore conducted a nested case-control study in which we surveyed a sample of approximately 2,500 women in LA County (half of whom gave birth to a low weight or preterm infant) approximately four months after delivery to collect additional information on these factors (the survey was funded by the National Institute of Environmental Health Sciences [NIEHS]). As part of our survey, we asked women detailed information about their residential history and commuting habits during pregnancy. Of the 6,374 women sampled from the cohort of births during 2003, we conducted interviews with 2,544 women (response rate of 40%). Of these 2,544 women, 1,319 (52%) worked outside of the home during pregnancy. The median reported (one-way) commute length was 9 miles (range 0.1–100 miles) and 20 minutes (range 1–350 minutes) and 180 women (~14%) reported commuting 60 minutes or longer. Eighty percent of women reported commuting by car, 13% by bus, 1% by metro, and 5.5% by walking. One woman reported getting to work by bicycle and 6 reported some combination of methods (e.g., car and train, bus and train). We also collected information on moves during pregnancy and commuting times and lengths for each home, thus, these percentages are based on all homes and distances reported. We calculated an average commute length and time for each woman who worked outside the home during pregnancy, weighting the distance (in miles and minutes) for each home by the time during pregnancy spent in each home. Women who did not work outside the home were given a commuting distance and time of zero (thus in our analyses they were always in the referent group along with women with very short commuting lengths and times). We observed a 47% increase in risk of delivering a preterm and LBW infant and a 55% increase in risk of delivering a LBW infant at term for women who commuted 45 minutes or more compared to those who commuted 5 minutes or less (OR=1.47, 95% CI=0.97–2.24 and OR=1.55, 95% CI=0.98–2.43, respectively). Adjustment for a number of important covariates did not change these estimates substantially. Effect estimates were greater when comparing women who commuted 60 minutes or more to work than those who commuted 5 minutes or less (OR=1.48,

95% CI=0.88–2.50 and OR=1.74, 95% CI=1.00–3.00 for preterm and LBW and term LBW, respectively), although estimates were reduced by approximately 10% with adjustment for important covariates and confidence intervals were wider due to the small sample size.

Conclusions

We observed an approximately 10–20% increase in risk of preterm birth (both normal and low weight) and term LBW in infants born to women potentially exposed to high levels of traffic-related air pollution, as represented by distance-weighted traffic density (DWTD) when focusing on births during 1994–1996. These risks appeared to be strongest for women whose third trimester fell during fall/winter months, who lived in high background air pollution areas, and/or who lived in more impoverished areas according to census block-group level indicators of socioeconomic status (SES). Although residential proximity to traffic did not appear to be associated with higher risks of term LBW or preterm-LBW birth in the later time period included in this analysis (1997–2000), residential proximity to trucks on freeways did appear to be associated with greater risks of these outcomes, especially preterm-LBW, during 1997–2000. This suggests more heavily polluting vehicles within the overall cleaner motor vehicle fleet, such as trucks, may have become more important for these outcomes in the later years. Our finding of positive associations between ambient CO concentrations and term LBW, preterm-LBW birth, and preterm birth in 1997–2000 suggests, overall, air pollution may still be harmful. We feel a more refined exposure assessment approach is needed at this point to arrive at further conclusions concerning the associations between residential proximity to traffic and adverse birth outcomes we have seen in our studies. One approach would be to develop an individual exposure model that takes into account exposure to indoor sources of air pollution and in-vehicle exposures, in addition to residential exposures to outdoor air pollution by accounting for concentrations within each of these microenvironments and time-activity patterns of pregnant women. Measurements of key traffic-related pollutants (CO, NO_2, UF) inside a sample of Los Angeles homes with varying levels of traffic in close proximity would provide needed information about typical at-home exposures and the influence of indoor pollution sources to overall exposure. Our results also indicate that in-vehicle exposures to air pollution while commuting during pregnancy may be important for low birth weight. We plan to analyze these data further and take into consideration the importance of type of commute (e.g., car versus bus, freeway versus surface streets), whether commuting distances vary by pregnancy period and if yes, the importance of this variation to the outcomes of interest, the importance of car age, and the importance of total time spent in a car including other in-vehicle activities besides commuting to work.

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Topic C: Studies of the Effects of Varying Spatial and Temporal Patterns of Ambient PM and Co-pollutants and Resulting Health Effects with Emphasis on the Role of Atmospheric Chemistry

Project 9: The Role of Quinones, Aldehydes, Polycyclic Aromatic Hydrocarbons, and other Atmospheric Transformation Products on Chronic Health Effects in Children (R827352C009)

Investigators: Edward Avol, Antonio Miguel, Arthur Cho, John Froines

Objective(s) of the Research Project: The overall purpose of this project was to provide exposure data on ambient organic pollutants in 12 communities participating in a long-term study of children's respiratory health. Two specific objectives were identified for this project: (1) characterize the annual levels and seasonal variability of selected PAHs, aldehydes, and quinones in ambient air in the study communities; and (2) apply the resulting data to ongoing

analyses of health and ambient pollution exposure, to help disentangle a previously identified and highly inter-correlated package of pollutants associated with decrements in human health. See Table 5 for a list of target analytes.

Table 5. Target Analytes of the Organics Sampling Study

PAHs	***Quinones***	***Carbonyls***
Naphthalene (NAP)	1,2-Naphthoquinone (1,2NQ)	Formaldehyde (FOR)
Acenaphthene (ACE)	1,4-Naphthoquinone (1,4NQ)	Acetaldehyde (ACD)
Fluorene (FLU)	9,10-Phenanthroquinone (PQ)	Acetone (ACE)
Phenanthrene (PHE)	9,10-Anthroquinone (AQ)	Acrolein (ACR)
Anthracene (ANT)		Propionaldehyde (PRO)
Fluoranthene (FLT)		Crotonaldehyde (CRO)
Pyrene (PYR)		Butanone (BUT)
Benz[a]anthracene (BAA)		Butyraldehyde (MET)
Chrysene (CRY)		Benzandehyde (BEN)
Benzo[*b*]fluoranthene (BBF)		Isovalerandehyde
Benzo[*k*]fluoranthene (BKF)		Valeraldehyde (VAL)
Benzo[a]pyrene (BAP)		o-tolualdehyde (OTO)
Indeno[*1,2,3-c,d*]pyrene (IND)		m-tolualdehyde (MTO)
Dibenz[*a,h]*anthracene (DBA)		p-tolualdehyde (PTO)
Benzo[*g,h,i*]perylene (BGP)		Hexaldehyde (HEX)

Background

In 1993, a multi-year study of public school children, CHS, was established in Southern California to assess the potential chronic health effects of ambient air pollution (Peters, et al., 1999). Twelve CHS communities were identified for study participation, based on their historic and predicted air pollution profiles, community demographics, and school district support. Over 6,000 4th, 7th, and 10th grade students were recruited, enrolled, and annually evaluated across these communities, to assess annual lung function growth, respiratory symptoms, and school-based absences caused by respiratory infection. To characterize local air quality, existing community air monitoring stations in each of the 12 communities were augmented with additional air sampling instrumentation, or a central air monitoring station was established, to provide continuous long-term information about ambient ozone, oxides of nitrogen, and particle concentration (as PM_{10} or $PM_{2.5}$ mass), as well as particle speciation ($PM_{2.5}$ acids, sulfates, nitrates, ammonium, elemental and organic carbon).

Several health-based manuscripts have reported on the observed associations between specific pollutants, or inter-correlated groups of pollutants, and various health outcomes measured in the CHS (Gauderman, et al., 2000; Gauderman, et al., 2004; McConnell, et al., 1999; Gilliland, et al., 2001; Avol, et al., 2001; McConnell, et al., 2002).

The co-linear behavior of several monitored pollutants made efforts to assess the relative importance of single pollutant measures with observed health outcomes difficult. In developing this component of the SCPCS's research program, the hypothesis was that measurement of specific organic compounds could provide alternative exposure metrics, less complicated by collinearity. In addition, the study was designed to provide data regarding the inter-community variability of organic pollutants of potential health importance, such as PAHs, primary and secondary aldehydes, and quinones, all of which are associated with the current urban living environment and motor vehicle emissions

Summary of Findings: Using an innovative sampler deployment approach to collect seasonal samples in 12 sampling locations with only three sets of instrumentation, field sampling was successfully performed in consecutive two-month deployments across all 12 CHS communities between 2001 and 2003. In the course of field operations, an improved sampling matrix was developed to successfully capture and stabilize particle and vapor-phase PAHs, aldehydes, and quinones in a multiple-media sampling matrix for 24-hour sampling intervals.

The collected samples were analyzed, edited, and developed into a cumulative database. Analyses were performed by both environmental researchers interested in the chemical interaction of pollutants in ambient air and by health researchers seeking to potentially disentangle previous associations among assorted respiratory health outcomes and a highly inter-correlated bundle of pollutants arising from energy combustion and vehicle emissions.

Achievements of Project Objectives

The collected data have been invaluable with regard to achievement of the exposure characterization objective. This work has produced a rich database on exposure to a set of organic compounds found in the ambient air of twelve Los Angeles area communities. As a result of this study, the interplay among ambient temperature, re-distribution of chemical species between gas and particle phases, and air toxics emissions associated with vehicle exhaust, energy combustion, and photochemistry has advanced substantially. Three manuscripts were published on these subjects (Eiguren-Fernandez, et al., 2004; Eiguren-Fernandez, et al., 2007; Cho, et al., 2004).

The project's second objective, to apply this data to health analyses to potentially de-couple previously observed relationships between a number of co-linear pollutants and respiratory health outcomes has also been achieved. Some associations between target analytes and two key respiratory health effect measures (lung function level and growth) have been observed. However, the analytical results developed thus far have not identified specific pollutants that provide explanatory power significantly beyond that of routinely monitored substances. In two-pollutant models assessing lung function, for example, the apparent effects of routinely collected pollutant data (such as ambient NO_2, $PM_{2.5}$ mass, elemental carbon, and acids) overshadowed the

effects of the carbonyls, quinones, and PAHs that were monitored for this study. Additional analyses assessing respiratory symptoms (such as bronchitis and wheeze) and asthma incidence are currently underway.

The research objectives of this project have, in large part, been fully realized. Ambient levels and shifts between vapor and particle phases due to temperature changes of ambient PAHs, carbonyls, and quinones have been characterized. The information collected in the course of the investigation has also suggested several additional lines of inquiry and future analyses.

Project's Significant Technical Details

Methods. Three sets of sampling instrumentation were purchased or assembled for field study applications, in conformance with the project proposal. Samplers were initially evaluated in a multi-day side-by-side comparison in Los Angeles to assure comparability. Samplers were deployed, for alternating two-month periods beginning in mid-2001 and continuing into Fall 2003. Sampling was performed in three CHS communities at a time, with collection beginning at midnight for a 24-hour continuous period once every eight days. Upon completion of seven sampling days (about two months' elapsed time), samplers were relocated to a second set of three study communities. There, sampling was performed in analogous fashion (e.g., the one-in-eight-day schedule), after which the samplers are re-calibrated and returned to the former three sampling sites for a repetition of the one-in-eight-day, two-month sampling protocol. In this manner, the three sets of samplers were used to collect speciated chemical data across six sampling sites each year, and data were collected at all 12 sampling locations over a two-year sampling period.

In each sampling location, two types of samplers were deployed. A Tisch Environmental Model 1202 Semi-Volatile Organic Compound (SVOC) and $PM_{2.5}$ sampling system and an in-house carbonyls sampler were used to collect PAHs, aldehydes, and quinones (see Table 5). PAH sampling was performed using a quartz filter/polyurethane foam (PUF)/XAD resin sampling matrix, while carbonyl sampling was accomplished utilizing a more conventional DNPH-based sampling cartridge. Additional sampling details have been reported (Eiguren-Fernandez, et al., 2004; Cho, et al., 2004).

Existing community monitoring stations provided supporting air monitoring information for several other pollutants of analytical interest (ozone [O_3], nitrogen dioxide [NO_2], PM mass [PM_{10} and $PM_{2.5}$], elemental carbon [EC], organic carbon [OC], and nitric/formic/acetic acids [acid]). Data from the routine sites were used in one of two ways, either to derive a 1994 through 2003 average level for pollutants of interest, or to develop a 2001 through 2003 concentration metric. The '94 to'03 readings were used to compare the relative health outcomes from analyses with those observed in a recent publication of CHS data (Gauderman, et al., 2004). The 2001 to 2003 calculations were used to provide a sampling interval more specifically appropriate to the time-frame of the PAH, carbonyl, and quinone measurements collected in the current study.

Health outcome data used in the analyses included indices of changes in two lung function tests: forced expiratory volume in the first second of exhalation (FEV_1) and maximal mid-expiratory

flow rate (MMEF). Changes in FEV_1 or MMEF have been previously shown to be significantly affected by air pollution in this schoolchildren population, and are thought to represent changes in large and small airways, respectively (Gauderman, et al., 2004; Gauderman, et al., 2000; Avol, et al., 2001). To qualitatively compare present analyses with those previously published results of long-term growth interest, only those subjects who entered the CHS as 4th graders (i.e., those whose lung function growth was tracked annually for the duration of the multiple-year CHS study) were included in the health analyses.

Data Analyses. Following editing and validation of the sampling data set, several analytical approaches were applied. For exposure assessment analyses, individual pollutant means, medians, and minimum/maximum values were tabulated by community and in total to assess summary distributions and consider possible correlations across the various pollutant metrics. Correlation tables were developed for all validated pollutants to identify possible unique contributors to any observed health outcomes. Data correlations were compared for observed values of PAHs, carbonyls, and quinones to both the 1994–2003 and 2001–2003 pollution metrics available from the CHS. One and two-pollutant models were then used to explore the possible additional explanatory value contributed by PAHs, carbonyls, or quinones over that provided by the previously available study pollutants in analyses of lung function endpoints.

A multi-stage modeling strategy was used to assess the relationship of PAHs, aldehydes and quinones to lung function measurements from children residing in the study communities and participating in a long-term health study (Gauderman, et al., 2004).

The first-stage model was a linear regression of each pulmonary function measure (log-transformed) on log height, body mass index (BMI), BMI^2, race, Hispanic ethnicity, doctor-diagnosed asthma, any tobacco smoking by the child in the last year, exercise or respiratory illness on the day of the test, and indicator variables for field technician and spirometer.

The second stage consisted of a regression of 48 (2 cohorts × 2 sexes × 12 communities) estimates of lung function growth over an eight-year period on the corresponding mean/median level of PAHs, aldehydes, and quinones in each community. The inverses of the first-stage variances were used as weights, and a community-specific random effect was included to account for residual variation between communities. Modification of the PAH effect by cohort was tested by including a cohort-by-PAH/aldehyde/quinone interaction term. This effect was found to be non-significant; hence all subsequent analyses were based on combined cohort effects. A similar procedure was followed to test for modification effect of PAH by sex. All subsequent models were adjusted for both cohort and sex unless otherwise specified.

Two-pollutant models were also tested by simultaneously regressing growth in lung function over the eight-year period on pairs of pollutants, of which one pollutant was a routinely monitored pollutant such as ambient NO_2, $PM_{2.5}$ mass, elemental carbon, and acids, and the other pollutant was one of the PAHs, aldehydes, or quinones measured in the current study.

Pollutant effect estimates were reported as the difference in the growth between the highest to the lowest pollution community, with negative sign denoting the detrimental effect with increasing exposure. Statistical procedures were performed using a commercially available

statistical package (SAS, Version 9). Statistical significance was defined by a two-sided alpha level of 5%.

Selected Results

Pollutant Levels. In the collected samples, virtually all of the total PAH mass was contained in the vapor phase. Vapor phase mass was dominated by naphthalene (NAP), which varied from about 60 ng/m^3 in lightly-trafficked rural communities to over 550 ng/m^3 in communities traversed by ~200,000 vehicles per day. During summer pollution episodes in urban sites, NAP concentrations reached 7 to 30 times the observed annual averages for those same locations.

Except for summer episodes, concentrations of low molecular weight (MW) PAHs showed small seasonal variations, with observed values about twice as high in winter. Similar concentrations of particle-phase PAHs were observed at all sites except for one rural coastal site, which was markedly lower. Benzo[*ghi*]perylene (BGP), a marker of gasoline exhaust emissions, showed the highest concentration among particle-phase PAHs, varying from 23 pg/m^3 in rural areas to over 230 pg/m^3 in communities containing high volumes of freeway traffic (hundreds of thousands of vehicles per day). Benzo[*a*]pyrene and indeno[*1,2,3-cd*]pyrene were found exclusively in the particle-phase, and they were much higher in urban sites (~ 40 to 100 pg/m^3) than in rural sites (~12 pg/m^3). Winter particle-phase PAHs were 2 to 54 times higher than summer levels. The data suggest that vehicle emissions are a major contributor to particle-phase PAHs in Southern California

Particle-phase PAHs were negatively correlated with mean air temperature in urban sites (r = -0.50 to -0.75). Cold/hot season ratios for PAHs in $PM_{2.5}$ averaged 5.7, with a maximum ratio of 54 calculated for data collected in Long Beach, a coastal community impacted by significant levels of primary emissions from vehicle traffic, commercial shipping, oil refineries, and population activities. These data underscore the importance of seasonal variation in atmospheric metrics such as ambient temperature, photochemical reactivity, and inversion layer height, as well as in gas-to-particle phase shifts of semi-volatile PAHs in ambient air.

Pollutant Correlations. With one exception, vapor-phase PAHs were essentially un-correlated with ambient ozone (as 24 hr, 10am to 6pm, or maximum value metrics), 24 hr NO_2, 24 hr PM_{10}, acids, elemental carbon (EC), or organic carbon (OC). Naphthalene was found to be moderately correlated with acids (r=0.643, p=0.02) and with NO_2 (r=0.610, p=0.035). In contrast, there were a number of strong correlations between the routinely monitored pollutants and particulate phase PAHs. Pyrene was correlated with acids (r=0.896, p<0.0001), pyrenes with 24 hr NO_2 (r=0.797, p=0.002), and pyrenes with 24 hr PM_{10} (r=0.707, p=0.01). Particulate acenapthene (r=.769, p=0.003), fluorene (r=0.821, p=0.001), and naphthalene (r=0.708, p=0.01) were all highly correlated with 24 hr O_3, and fluoranthene was found to be highly collinear with 24 hr NO_2 (r=0.869, p=0.0002).

A number of aldehydes (formaldehyde, acetaldehyde, acetone, protonaldehyde, and butanone) were very highly correlated with ambient acids, 24hr NO_2, 24hr PM_{10}, OC and EC (coefficients of 0.7 to 0.9, and p-values typically less than 0.001). The significant correlations suggest that the

carbonyl data will not be able to unravel observed health associations among the highly-correlated routinely monitored pollutants.

Among the quinones monitored, particle-phase 1,4 naphthoquinones were strongly correlated with acids (r=0.782, p=0.003), 24 hr NO_2 (r=0.627, p=0.029) and marginally correlated with EC (r=0.561, p=0.058) and 24 hr PM_{10} (r=0.567, p=0.055). Particle-phase phenanthroquinones were found to be correlated with acids (r=0.630, p=0.028). No other particle or gas-phase quinones were correlated with the routinely monitored pollutants.

The correlations and relationships between and among pollutants measured were similar when either the 1994 through 2003 community pollution exposure averages or the 2001 through 2003 community pollution exposure averages were used. The latter were more specific to the current organic sampling study timeframe, but both provided similar and consistent results. This observation provides validation for comparing PAH, aldehyde, and quinone exposures to the longitudinal health outcomes derived from the ten-year cumulative health investigations.

Health Analyses. Two-pollutant models comparing pollution level and lung function growth rate changes were run, to assess the relative importance of each individual organic pollutant monitored in the current study compared to the pollutant data set (i.e., O_3, NO_2, PM_{10}, $PM_{2.5}$, EC, OC, and acids) previously available from the health study communities.

In two-pollutant models with O_3 and the compounds monitored for this study, there were sporadic tests achieving statistical significance. Of those, a few showed significant changes for the organic pollutants monitored. It should be noted that ozone has typically not been associated with statistically significant decrements in lung function growth rates in prior CHS health analyses. When there were significant results (for acetaldehyde, acetone, naphthalene, and phenanthrene, for example), these test pollutants were significant over ozone and showed changes in the predicted (i.e., negative) direction.

However, two-pollutant models with pollutants that have previously been linked to decrements in lung function growth rates showed a distinctly different pattern. In comparisons of NO_2 with the PAHs, quinones, and aldehydes, NO_2 was found to be significant in nearly every comparative case. In models with $PM_{2.5}$ and each test PAH, quinone, or aldehyde measured, $PM_{2.5}$ was found to be the more significant modeled pollutant in most every comparative case.

These results suggest that some of the PAHs, quinones, and aldehydes measured in the study appear to be associated with decrements in lung function growth. However their added explanatory power toward the endpoints of the CHS study is minimal compared to the decrements already identified by associations with the inter-correlated package of pollutants previously monitored (including NO_2, $PM_{2.5}$, EC, and acids). This suggests that, with respect to lung function growth rate indices, the PAHs, quinones, and aldehydes measured have thus far not helped to disentangle the "package" of pollutants previously found to be associated with the lung function growth indices examined. Some routinely monitored pollutants such as NO_2 and $PM_{2.5}$ appear to be either more important or measured with less error than the PAHs, quinones, or aldehydes tested in this study in accounting for the observed effects.

Project's Significant Technical Details

Impurities in the PUF portion of the original sampling matrix led to persistent difficulties in laboratory quinone derivatization procedures. To avoid these problems, a revised sampling matrix that included additional XAD resin was utilized in the second year of sampling, i.e., in the second set of six CHS communities of Long Beach, Lancaster, Santa Maria, Lake Elsinore, Alpine, and Lake Arrowhead. This change dramatically improved the ability to quantify vapor-phase quinones. A few of the targeted collection species' data (anthroquinone and acrolein) had to be invalidated, due to concerns about collection validity and artifact formation on the collection media.

Conclusions

Ambient air samples were collected in 12 Southern California communities to assess seasonal variability and annual estimates of 15 PAHs, four quinones, and 15 aldehydes of environmental and health concern. Analyses revealed that:

1. Virtually all of the total PAH mass was found in the vapor-phase and vapor phase PAHs were dominated by naphthalene.

2. Vapor-phase PAH concentrations were essentially uncorrelated with those of the more commonly measured pollutants (O_3, NO_2, PM_{10}, EC, OC, ambient acids).

3. Several particle-phase PAHs and aldehydes were strongly correlated with more commonly measured pollutants (O_3, NO_2, PM_{10}, EC, OC, ambient acids).

4. Particle-phase PAH levels were similar across most of the study sites.

5. Particle-phase PAH levels were 2 to 54 times higher in winter than summer.

6. Particle-phase PAH concentrations were negatively correlated with mean air temperature.

7. In two-pollutant models assessing decrements in lung function growth rate indices (FEV_1 and MMEF), the PAH, aldehyde, and quinone constituents did not generally provide additional clarification in the health analyses over NO_2 or $PM_{2.5.}$

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Project 10: Novel Method for Measurement of Acrolein in Aerosols (R827352C010)
Investigator: Judith M. Charles

Objective(s) of the Research Project:

Hypothesis

The overall objective of the project is to develop a method to measure acrolein, and other carbonyls that either are directly emitted from motor vehicles or are photooxidation products of

hydrocarbons in motor vehicle exhaust (e.g., crotonaldehyde, hydroxyl acetone, glycolaldehyde, methyl glyoxal, glyoxal) that is accurate and precise, and that affords a short sampling time.

Objectives

The objective of this project was to develop a new method to measure acrolein and other toxic carbonyls in air that affords part-per-trillion detection limits and short sampling times (10 minutes). The proposed method relies on using a mist chamber to sample carbonyls, followed by detection of the compounds by using derivatization along with gas chromatography/mass spectrometry (GC/MS).

Summary of Findings: Initial work was conducted to explore whether carbonyls could be sampled into a mist chamber by using an aqueous bisulfite solution, and whether the carbonyls could be analyzed by releasing the carbonyl-bisulfite adduct and then derivatizing the "free" carbonyl with 0-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) prior to detection by using GC/MS. Specifically, experiments were conducted to: 1) establish the formation of carbonyl-bisulfite adducts by measuring the formation constants (k_f) for formaldehyde, methyl glyoxal, acrolein, glyoxal, methacrolein, crotonaldehyde, hydroxy acetone and glycolaldehyde, 2) investigate the effect of pH on PFBHA derivatization of the carbonyls in the presences and absence of bisulfite, and 3) investigate the effect of bisulfite concentration on PFBHA derivatization. Once optimum conditions were determined for formation and derivatization of the carbonyls, we compared the collection efficiency (CE) in the mist chamber using an aqueous solution, a 0.001M bisulfite solution, a 2mM PFBHA solution and a 2mM pentafluorophenylhydrazine (PFPH) solution. The collection efficiency is a measure of the efficiency of the mist chamber to capture the carbonyls is the first mist chamber, using 2 mist chambers in series.

The k_f of the carbonyls were determined at pH=5.0 using pseudo-first order kinetics. The k_f for formaldehyde was 15.15 ± 1.36 $M^{-1}s^{-1}$ which is in close agreement with the literature value of 12.83 $M^{-1}s^{-1}$, thereby validating the analytical approach. The formation constant for acrolein was 0.73 ± 0.10 $M^{-1}s^{-1}$ with a 96% yield in 10 minutes. These k_f and the k_f obtained for the other compounds establish the formation of carbonyl-bisulfite adducts at pH=5.0 with >80% yields in 10 minutes. Since samples will be collected in the field, and then prepared for analysis in the laboratory, we also explored the stability of the bisulfite adducts over a 6 day period. The effect of pH on PFBHA derivatization was explored at pH=1, 5 and 12 in the absence of bisulfite. The results were consistent with previous data that demonstrate that PFBHA derivatization occurs at neutral and acidic, but not basic pH. The experiment was repeated at pH=5.0 in the presence of 0.05 M bisulfite. The concentration of the PFBHA derivatives was lower in the presence of bisulfite than in the absence of bisulfite, indicating that the bisulfite hinders PFBHA derivatization of the carbonyls. Further experiments established that derivatization was also compromised at 0.005 M and 0.001 M bisulfite concentrations, with higher concentrations of derivatives being formed at the lowest bisulfite concentration. The finding that bisulfite hinders or interferes with PFBHA derivatization was significant and may be a deterrent to using an aqueous solution bisulfite as the collecting medium in the mist chamber. Comparison of the collection efficiency for acrolein, methacrolein, methyl vinyl ketone, crotonaldehyde, glyoxal and methyl glyoxal using water, a 0.001 M bisulfite solution, a 2 mM PFBHA solution and a 2

mM PFPH solution demonstrated this occurrence. Overall the collection efficiency followed the order of water < PFBHA solution < 0.00 1M bisulfite solution < PFPH solution. For example for acrolein, the collection efficiency in water, a 0.001 M bisulfite solution, a 2 mM PFBHA solution and a 2 mM PFPH solution was 0.18±0.04, 0.448±0.13, 0.19±0.07, and 0.89±0.07, respectively. The reason that the collection efficiency for PFPH is higher than PFBHA is not clear, but may be due to greater water solubility and lower volatility of the hydrazone derivatives, and faster and more expedient derivatization of the carbonyls using PFBHA compared to PFPH (see Figure 9).

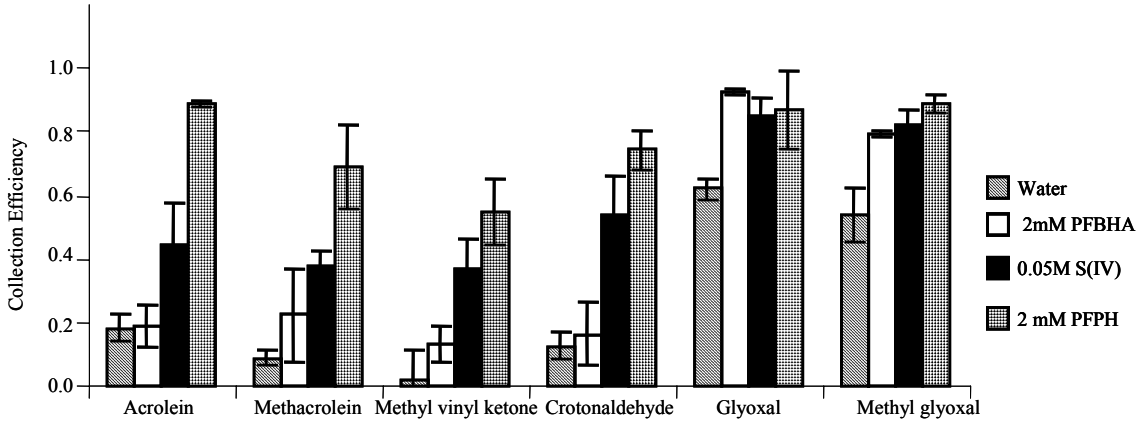

Figure 9. Comparison of Collection Efficiencies of Acrolein and Other Carbonyls Using Different Solutions in a Mist Chamber

In view of the established k_f for the carbonyls, and the water-solubility and stability of the carbonyl-bisulfite adducts, the lower collection efficiency in the presence of the bisulfite solution compared to using PFPH is likely a result of poor derivatization of the carbonyls with PFBHA in the presence of bisulfite. The difference between the collection efficiency when water is used as the collection media vs. an aqueous solution of PFPH indicates that formation of the derivatives is critical to efficiently capturing the carbonyls.

Previous research conducted in our laboratory demonstrates the ability of a 2 mM solution in a mist chamber to sample carbonyls with Henry's law constants < 10^3. To sample less polar carbonyls, such as acrolein, a 0.001 M bisulfite or a 2 mM PFPH solution can be used. Although the use of the bisulfite solution to collect the carbonyls and form carbonyl-bisulfite adducts is an attractive approach due to the water solubility of carbonyl-bisulfite adducts, PFBHA derivatization of the carbonyls is hindered in the presence of S(IV). For this reason, if bisulfite is used in the mist chamber to collect the carbonyls, we suggest that further research be conducted to investigate the direct detection of the carbonyl-bisulfite adducts. The high collection efficiencies for acrolein (CE=8.0), and overall good collection efficiency (CE > ~6.0) for methacrolein, methyl vinyl ketone, crotonaldehyde, glyoxal and methyl glyoxal using a 2 mM PFPH solution in the mist chamber indicates that use of PFPH along with detection by using GC/MS will afford sensitive detection of acrolein and other toxic carbonyls.

Project 11: Off-line Sampling of Exhaled Nitric Oxide in Respiratory Health Surveys (R827352C011)
Investigators: Henry Gong, William Linn

Objective(s) of the Research Project: Delayed offline measurement of exhaled nitric oxide (eNO) has important applications in environmental health screening studies where direct online measurement can be impractical. However, the use and application of delayed offline eNO has been limited by the instability of stored breath samples. Our goal was to develop a practical method for off-line measurement of eNO that can be applied in larger multiple-site epidemiologic surveys, with reliable preservation of exhaled breath samples for analysis at one central laboratory.

Summary of Findings:

Description of Research

Exploratory experiments indicated that breath could be collected satisfactorily in commercial eNO sampling bags (Sievers Instruments, Boulder, CO) or in structurally similar toy balloons made of aluminized polyethylene terephthalate film (Mylar or equivalent). We used both sampling bags and balloons with a Bag Collection and Sampling Kit (Sievers Instruments) that allowed a subject to take a vital-capacity inspiration of relatively NO-free air and immediately exhale it at a measured flow rate near 100 mL/sec. Exhaled air was allowed to escape for the first 3–6 sec to clear deadspace, then a sample of 0.5 to 1.5 L was collected. After preliminary experiments to optimize the procedure, we obtained 185 air samples, including:

- breath from 38 nonsmoking volunteer subjects (10 adults, 22 high school students, and 6 children aged 9–13, including individuals with asthma or other chronic cardiorespiratory conditions),
- ambient air over a range of NO pollution conditions,
- air filtered by the breath collection apparatus (like the air inhaled by subjects during tests), and
- commercial zero air free of NO.

Samples were collected in our laboratory and at 5 field locations (3 schools, 2 homes) in metropolitan Los Angeles. Each sample was measured repeatedly with a chemiluminescent NO analyzer (Sievers NOA Model 280i) over a period of 1–7 days. A calibration check with zero and span gas was performed within 20 min of each sample measurement. Samples were stored at 42, 22, 6, or -14 degrees Celsius to evaluate effects of temperature on stability. Certain samples were stored in largely NO-free atmospheres, and compared with others stored in NO-polluted ambient air, to test effects of leaks or permeation.

Regression analyses were performed to evaluate samples' changes in NO concentration over time and to identify factors that influenced those changes, including sample source, initial concentration and storage temperature.

Findings

We found both positive and negative changes in NO concentration during eNO sample storage, depending upon temperature, initial concentration and reaction with ambient air. For example, NO in breath samples stored at 22 degrees increased with time in approximately linear fashion; greater increase was noted for samples stored at 42 degrees. In contrast, storage at -14 degrees had a negative effect. Samples with initially high NO tended to show decreases over time, while samples initially low in NO showed very slow increases over time at 6 degrees. Variability within subjects was similar to that reported from previous studies of on and immediate offline measurements.

The findings of increased NO in samples with low or zero initial concentrations support an earlier paper in which it was suggested that aluminized Mylar containers can desorb NO into collected samples. In our hands, this desorption appears to be temperature sensitive.

Conclusions and Recommended Sampling Protocol

The study was successful in developing procedures suitable for studies that require large-scale collection of breath samples at field locations, and measurement of NO in a central laboratory. We found that numerous sources of variability may influence the delayed offline measurement of eNO. The resulting errors may differ in direction as well as magnitude, depending upon the collection and analysis system. Our system generally was associated with gain of NO at or above room temperature and loss of NO at lower temperatures. Given these findings, the key requirements for a successful study that were identified include refrigeration of Mylar sampling containers after collection and standardization of the time interval between collection and analysis. Based on our findings and information from published literature, we recommend the following for off-line eNO measurement in the Children's Health Study and similar surveys:

1. The Sievers Bag Collection and Sampling Kit and Sievers NOA 280i analyzer, or equivalent equipment, should be used. The manufacturer's recommended procedures should be used, except that an abbreviated zero and span check should be performed at least every 20 min during analysis, and sample readings should be adjusted according to the zero/span check results closest in time.

2. The subject should perform a vital-capacity inspiration through an NO-reducing filter, followed immediately by a flow-controlled vital capacity expiration. Expiratory flow should be standardized at 100 mL/sec, as read by the sampling kit's expiratory pressure gauge with appropriate allowance for altitude. Deadspace gas must be discarded, so collection should begin between 3 and 6 seconds after the start of expiration.

3. Two samples should be collected from each subject. One initial practice maneuver with no collection should be allowed, at least for younger children.

4. The Sievers 1.5-L sample bag, washed 3 times with zero-NO gas and evacuated per manufacturer's instructions, is the optimum sample container, reusable many times with careful handling. Bags must be individually identified, and the identity code must be recorded at each sample collection to facilitate quality review. Alternatively, aluminized Mylar self-sealing balloons may be used once and discarded.

5. The same type of sample container should be used throughout a study.

6. For each collected sample the technician should observe and code the expiration as satisfactory, slow, fast, or erratic.

7. The time from collection to analysis, and the temperature during that time, must be similar for all samples. One possible strategy is to analyze samples 24 ± 4 hr after collection, keeping them refrigerated at approximately 5° C, or in heavily insulated containers surrounded by ice, in the interim.

8. Insofar as practical, the analyst should be blind to the origin of each sample. If measurement is by the Sievers automated recording procedure, the point estimate for a sample should be the mean determined from a plateau of concentration with duration ≥15 sec. For manual measurement, at least three independent readings should be taken between 15 and 25 sec after starting the flow of sample air, and their median should be the point estimate for the sample. The mean reading from two samples should be the point estimate for a subject. If the two samples differ by >30% of the larger reading or by >3 ppb, whichever is greater, the data should be discarded as unreliable.

The work was published in:

Linn WS, Avila M, Gong H. Offline measurement of exhaled nitric oxide: sources of error. *Archives of Environmental Health* 2004;59(7):1-7.

Project 12: Controlled Human Exposure Studies with Concentrated PM (R827352C012)
Investigator: Henry Gong

Objective(s) of the Research Project: The objectives of this project were as follows:

1. Perform controlled exposures of human volunteers to typical Southern California urban ambient atmospheres polluted by particulate matter (PM), using ambient particle concentrators. The exposures were designed to represent realistic combinations of relatively high PM concentrations and vulnerable people. The different exposure projects addressed the three key PM size ranges (F, C, and UF) in a metropolitan location with heavy primary PM pollution and secondary photochemical PM pollution. The volunteer subjects included healthy adults (under age 50), asthmatic adults, healthy elderly adults, and elderly adults with chronic obstructive pulmonary disease (COPD).

2. Measure short-term health effects of the above exposures, using the most sensitive practical (i.e., relatively noninvasive) measures of cardiopulmonary health and systemic inflammation. The acute biological endpoints to be measured were selected for relevance to recent epidemiological studies and included symptom inventories, clinical respiratory function (e.g., forced expiratory spirometry, pulse oximetry), airway inflammation (as inferred from cytology and biochemistry of induced sputum), systemic inflammation, and hemostasis (as inferred from biochemistry of peripheral blood), and cardiac rhythm alterations documented from continuous ambulatory electrocardiograms (ECG).

These clinical efforts were among the first in the country to apply concentrator technology capable of concentrating different PM modes. The School of Engineering of the University of Southern California provided significant environmental monitoring and concentrator support during the studies. All exposures were performed at the base laboratory location (Downey, CA), which is heavily influenced by local and regional primary motor-vehicle pollution, as well as with some emissions from stationary sources and ships. Thus, we were able to focus and study intensively the health effects of ambient PM at one dedicated and characterized impacted location.

The SCPCS-supported studies (in combination with immediately preceding and concurrent efforts primarily funded by the EPA through other grants) yielded the health effects investigations and publications summarized in Table 6. These efforts were complementary and shared important components so that comparisons and understanding of the results could be efficiently derived. Each study was approved by the local institutional review board and each subject provided signed informed consent prior to his/her participation. All studies followed essentially the same experimental protocol, involving 2 hours of exposure to the scheduled PM mode and control (filtered air alone) in a single-person exposure chamber (a modified body plethysmograph interfaced to the appropriate particle concentrator). Moderate ergometer exercise was performed for 15 min of each half hour. Holter ECG was continuously recorded for about 24 hr, beginning about 1 hr before the experimental exposure. Most measures of health status were ascertained just before and immediately after the exposure period, again 4 hr after exposure ended, and again on the following morning (about 22 hr after exposure ended). Sputum induction to assess airway inflammation was performed only on the morning following each exposure.

Table 6. Summary of PM Concentrator/Human Exposure Studies Associated with the SCPCS

Atmosphere, Mean Concentration	**Subjects**	**Primary Sponsor**
$PM_{2.5,}$ 174 $\mu g/m^3$	12 healthy + 12 asthmatic adults	Health Effects Institute
$PM_{2.5}$, 194 $\mu g/m^3$	6 healthy elderly + 13 elderly with COPD	SCPCS
$PM_{2.5}$, 203 $\mu g/m^3$ + NO_2, 0.4 ppm	6 healthy elderly + 18 elderly with COPD	EPA-STAR
$PM_{10-2.5}$, 157 $\mu g/m^3$	4 healthy + 12 asthmatic adults	SCPCS

$PM_{0.1}$, 145,000 particles/ml	17 healthy + 14 asthmatic adults	SCPCS

Summary of Findings:

Healthy and Asthmatic Adults Exposed to Fine Particles ($PM_{2.5}$)

A two-hour exposure to F was associated with mixed exposure-related unfavorable effects. This might be expected given the short duration, moderate PM exposure concentration (25–50% of normal levels for that neighborhood) and small group sizes. We were able to detect differences by exposure group in some acute cardiovascular, circulatory and central airways responses.

Conventional lung function tests and routine hematologic measurements did not show statistically significant ($P < 0.05$) changes in either healthy or asthmatic subject groups after F exposure, relative to filtered-air control conditions. PM-related decreases of columnar epithelial cells were observed in the sputum of subjects from both groups, and this finding was statistically significant. Both respiratory rate and volume decreased in the exposed group, but respiratory symptoms were not significantly altered. Ventilation differences were corroborated by significant differences in average heart rate recorded during exposure. Both groups showed slight changes in certain mediators of blood coagulation and systemic inflammation, i.e., soluble intercellular adhesion molecule-1, interleukin-6, and factor VII. Symptoms related to cardiac function increased slightly with PM exposure. Heart rate variability data suggested a slight increase in parasympathetic influence on the heart during or after PM exposure, while sympathetic influence was unchanged or slightly decreased

Individual exposure concentrations varied by a factor of 2 in terms of total PM mass, and by larger factors when exposure to specific components was assessed, e.g., nitrate, sulfate, elemental carbon, iron. To explore effects of individual variation on results, numerous exposure-response relationships (i.e., correlations of individual change in a health endpoint with individual difference in a specific atmospheric component between PM and control studies) were calculated. Most were non-significant, as expected given the relatively low statistical power available from a small subject group. Among the exposure variables tested, sulfate showed the largest number of statistically significant relationships with health endpoints. Increasing sulfate concentration was associated with decreasing respiratory rate and minute volume during exposure, and decreased forced expiratory function post-exposure, although the overall difference in respiratory function between exposure and control was non-significant. The ratio of parasympathetic to sympathetic influence on heart rate, in addition to its significant overall difference between exposure and control, showed a significant relationship to exposure PM mass concentration.

Elderly Subjects With and Without COPD Exposed to Fine Particles ($PM_{2.5}$)

In the studies of elderly volunteers, we deliberately recruited an excess of individuals with COPD compared to those in good health. We expected that those with COPD would be more likely to show unfavorable effects, but also would show greater variance in their responses, thus requiring larger numbers to improve statistical power. This expectation was not realized: if

anything, the healthy subgroup showed more evidence of exposure effects. We found no significant changes in symptom reports, lung function, or assays of induced sputum after F exposure, relative to control. Modest but statistically significant oxyhemoglobin desaturation (as measured by fingertip pulse oximetry) was observed immediately after and 4 hr after exposure. The mean loss of oxygen saturation was significantly larger in healthy relative to COPD subjects. Individual oxyhemoglobin saturation changes were significantly related to individual exposure mass concentrations, but in the counter-intuitive direction: the subjects with higher exposure concentrations tended to have less negative changes in saturation. Peripheral blood basophils increased after exposure in healthy but not in COPD subjects. Both groups showed slight increases in red cell counts 1 day after filtered air exposure, but not after F exposure. Heart rate variability between 4 and 24 hr after F exposure was significantly decreased in healthy subjects; this finding was not observed in COPD subjects. The incidence of supraventricular and ventricular ectopic heartbeats varied between F and filtered air studies. Supraventricular ectopic incidence was low in healthy subjects in filtered air, and doubled in fine particle exposures. The supraventricular and ventricular ectopic incidences were higher overall in COPD subjects, but decreased in F exposures. The overall pattern of statistically significant findings was highly discordant between these elderly subjects and the younger adults studied earlier.

Separate and Combined Exposures to Fine Particles and Nitrogen Dioxide in Elderly Subjects With and Without COPD

This extension of the above study determined whether a gaseous pollutant that commonly accompanies primary particulate pollution—NO_2—would appreciably enhance unfavorable effects of particulate exposure. Most respiratory responses were again non-significant in exposures to F and NO_2, separately or combined. However, the maximal mid-expiratory flow, a measure influenced by peripheral airway patency, showed a small but statistically significant decline in healthy subjects exposed to F, whether or not NO_2 was present. F exposures were again associated with a small significant decline in arterial oxygen saturation, more pronounced in healthy than in COPD subjects. This response was not significantly affected by NO_2. Also, F exposures were associated with decreased percentages of columnar epithelial cells in induced sputum. Thus, it appeared that F was the primary pollutant causing acute health effects in this setting.

Healthy and Asthmatic Adults Exposed to Coarse Particles ($PM_{10-2.5}$)

The results of C exposure suggest that the responses are primarily systemic in nature, rather than localized to the respiratory tract. Small but statistically significant increases in heart rate and decreases in heart rate variability were observed, consistent with the decrease in heart rate variability observed in elderly adults exposed to F in the previous study, suggesting altered cardiac autonomic function. Those changes were larger in healthy than in asthmatic subjects. There were no significant changes in the incidence of ectopic heartbeats or exhaled nitric oxide between PM and filtered-air exposures. Relative to filtered-air exposures, C exposures did not elicit significant changes in respiratory function measurements, symptom reports, arterial oxygen saturation, or measures of airway inflammation. In addition to induced-sputum assays, a new tool to evaluate airway inflammation was made available by SCPCS support and initiated during this study, the exhaled nitric oxide (NOe) analysis.

Healthy and Asthmatic Adults Exposed to Ultrafine Particles ($PM_{0.1}$)

Relative to control exposures, UF particle exposures were associated with small but statistically significant disturbances of arterial oxygen saturation, forced expiratory flow rates (observed the day after exposure but not immediately after exposure), and low-frequency (sympathetic) heart rate variability. These responses were not significantly different in healthy and asthmatic subjects. Exhaled nitric oxide increased after UF particle exposure in healthy subjects, possibly suggesting airway inflammation, but decreased (from a higher baseline level) in asthmatic subjects, possibly suggesting improvement in preexisting airway inflammation. There were no significant differences in sputum cell counts between UF exposures and controls. Between individuals, decreased low-frequency heart rate variability correlated significantly with increased particle count in the exposure. Also, symptom score increases during and immediately after exposure showed a significant positive correlation with particle count. These associations were not significantly different in healthy and asthmatic subjects. Blood and sputum biochemistry data are available for only the initial 15 of 31 subjects. (The remaining biochemical analytical results are pending and are being kindly donated to this project by EPA laboratories.) The available blood data suggest that UF exposures did not affect blood coagulation properties or systemic inflammation, although the complete dataset could reveal statistically significant changes.

Discussion

More than 100 individual exposures to one or another size fraction of ambient particulate matter, typically concentrated to 8–10 times the usual ambient level, were conducted in this project. This experience is unique and demonstrates the overall feasibility and safety of clinical PM exposure studies using concentrator methodology. No major or clinically significant unfavorable responses have been observed in the exposures with the three major PM size fractions. This contrasts with the clinical experiences with ozone and sulfur dioxide, the pollutants from which the 2-hr, intermittent exercise investigative protocol was originally developed. For both gaseous pollutants, clinically obvious individual respiratory responses, as well as consistent patterns of statistically significant physiologic and symptom changes, can be observed at exposure concentrations within or modestly above the ambient range. Both here and in other laboratories performing human exposure studies with CAPs, statistically significant effects have been found in small groups of subjects, but the effects have been generally modest, and inconsistent across different studies. One reasonably consistent factor is that PM effects show up primarily in the cardiovascular rather than the respiratory system; cardiovascular effects are also found to be the most consistent outcomes in epidemiologic studies of PM. Thus, the concentrator studies have provided a basis for supporting evidence for systemic inflammation and cardiovascular effects frequently observed in epidemiological associations with PM. The PM concentrator studies in this project have generally not provided clear evidence for direct acute respiratory effects, although others have reported mild lung inflammation from bronchoalveolar lavage.

It is necessary to consider the limitations of the controlled-exposure studies described here and epidemiologic studies that might contribute to this lack of coherence. On the epidemiologic side, one possibly important limitation is lack of attention to weather factors, other than temperature,

that might contribute both to PM pollution and to cardiovascular stress. In laboratory-based exposure studies, small samples are an obvious inherent limitation, exacerbated by lack of uniform exposure and standard monitoring techniques, which makes it difficult to pool data across multiple studies. Another issue is the limited ability to control intercurrent ambient exposures (much more of problem with PM than with ozone or sulfur dioxide). A typical subject's inhaled dose of ambient particles might be increased by no more than a factor of 2 on an experimental PM exposure day, and reduced by no more than 10% on a filtered-air control day. Still another challenge is the combination of secondary stresses (confinement, noise, lack of precise temperature control) inherent in particle-concentrator-based exposure protocols, which might mask subtle responses to PM. Regarding motor-vehicle-derived PM, it may be possible to bridge some remaining gaps in understanding through controlled exposures while riding in heavy traffic, contrasting the responses to unaltered on-road pollution and to pollution with PM carefully filtered out. Such a study, drawing upon the experience gained in this project, is now in progress.

Conclusions

The body of evidence concerning acute effects of ambient PM inhalation has been considerably expanded by this project and by concurrent work with ambient particle concentrators in other laboratories. However, the extent to which PM contributes to increased morbidity and mortality on high-pollution days, the specific components of PM that present the greatest health risk, and the physiological/biochemical mechanisms by which they act, are still incompletely understood. The most consistent observation from this project and similar studies thus far is that circulatory effects are more likely expressed than respiratory effects, despite the fact that the respiratory system is most directly exposed to PM and other inhaled toxicants.

Project 13: Particle Size Distributions of Polycyclic Aromatic Hydrocarbons in the LAB (R827352C013)
Antonio H. Miguel, Constantinos Sioutas, Arantza Eiguren-Fernandez

Objective(s) of the Research Project: While epidemiological studies have firmly established that F can adversely affect the health of exposed individuals, even at exposure levels at or below the current ambient air standard, several questions regarding F toxicity remain elusive, including:

1. Which chemical component(s) or class of components of F are the most hazardous?

2. In addition to atmospheric concentration, what other properties of F are important?

3. What role does particle size play in F toxicity?

4. How is the toxicity of F affected as it mixes and reacts with gaseous air pollutants and free-radicals during advective atmospheric transport?

Beginning with the work of Ferin (1992), firmly establishing the existence of dramatic differences in pathogenicity resulting from different particle sizes of the same material, laboratory and field studies reported over the last decade firmly demonstrated that the size of

atmospheric particles determines: (1) the number of particles deposited at a tissue target, (2) where the particles deposit in the human respiratory system, (3) which aerodynamic mechanism governs their deposition in the respiratory tract, and (4) their atmospheric reactivity and life-times.

Particle size, concentration, and chemical composition are the aerosol properties that are among those likely to be most important to both chronic and acute toxicity. If we want understand, for regulatory purposes, how toxin-containing airborne particles are transported and transformed, we need to acquire detailed information of their particle size, concentration, and chemical composition, during all seasons of the year, both near their emission sources and in locations where humans are exposed to the airborne aerosol.

The major objective of this research project, complementing other SCPCS activities described elsewhere in this Final EPA report, was to acquire information on the effects of season and location on the concentration, and the size distribution of PAHs (defined by the U.S. EPA as Priority Pollutants) collected in Southern California communities. Detailed size resolution in the UF and Aitken (diameter <50 μm) size ranges constituted the ultimate foci of these studies.

Summary of Findings:

Achievement of Project's Objectives

We developed GC-MS and HPLC-selective fluorescence methods to measure all 16 U.S. EPA Priority Pollutant PAHs present in source and ambient air samples (Pereira, et al., 2001; 2002; Eiguren-Fernandez, et al., 2003). Using the NIST standard reference material (SRM) 1649a, which contains certified concentrations for several PAHs, our extraction and quantification procedures provided overall analytical precision of 4.2% and extraction recovery efficiencies ranging from 92 to 97% for all PAHs. We also participated in the development and validation of a GC-MS derivatization-based method used to quantify quinones in diesel exhaust particles, and ambient $PM_{2.5}$ samples collected in the LAB and other Southern California locations (Cho, et al., 2004).

We went on to evaluate the extent of sampling artifacts that might occur during the collection of size resolved semi-volatile and particle-phase PAHs using MOUDI impactors with and without an annular denuder (Eiguren-Fernandez, et al., 2003).

Between 2001 and 2003, we measured the seasonal and spatial variation of F and vapor-phase PAH concentrations at all 12 communities participating in a multi-year chronic respiratory health study of schoolchildren in the CHS. The communities were geographically distributed over two hundred kilometers, extending from coastal Central California through coastal Los Angeles, inland to Riverside and San Bernardino counties, and south into Eastern San Diego County (Eiguren-Fernandez, et al., 2004; Miguel, et al., 2004).

Concurrently with the development and validation of accurate and sensitive methods for PAH quantification, we measured, during all seasons, the particle size distributions of 12 priority pollutant PAHs, concurrently with elemental carbon (EC), organic carbon (OC), sulfate (SO_4^{2-}),

and nitrate (NO_3^-) size distributions. Samples were collected from October 2001 to July 2002 in Claremont, CA, a receptor site located about 40 km downwind of Central Los Angeles.

Our most recent observations of the size distributions of twelve target PAHs in the 10 nm< Dp<2.5 μm size range, showed that, overall, regardless of vapor pressure, the PAH masses in each of the differential fractions (10–18nm, 18–32nm, and 32–56nm) are larger in the smallest fraction (10–18nm) than that in the two larger fractions (Miguel, et al., 2005).

Given our results that naphthalene (identified by the U.S. EPA as a hazardous air pollutant, and listed in the 2002 State of California's Proposition 65 program as a substance known to cause cancer) constitutes about 95% of the measured PAH mass, together with several SCPCS researchers, we carried out new field measurements of the naphthalene-to-benzene ratio at the busy Sepulveda Tunnel (under LAX) in Los Angeles, to support an advanced modeling study to quantify population exposure to the emissions of naphthalene throughout Southern California (Lu, et al., 2005).

The studies related to the toxicity of PAHs and quinones, and their concentration in diesel exhaust particles (DEP) used in *in vitro* experiments (Li, et al., 2000; 2004; Cho, et al., 2005) are reported in detail elsewhere in this Final Report.

Project's Significant Technical Details

Analytical and Sampling Methods Development. The use of annular denuders was recommended in the literature as a means of reducing "sampling artifacts." The hypothesis was that, by removing vapor-phase species before particle-phase collection, and then capturing any of the same vapor-phase species that remained after particle-phase collection, the effects of sampling artifacts could be corrected by comparing the levels of the species found associated with the particle-phase and that captured downstream. Our results showed that at a source and a receptor area in the LAB, using either sampling system, the size distributions obtained were similar for PAHs found in the particle-phase, but different for the semi-volatiles. At a central Los Angeles site, the largest PAH fraction was found in the UF size range, typical of primary emissions. At the downwind location, the largest fraction was in the accumulation size range, consistent with an "aged" aerosol. We concluded that sampling with the regular MOUDI configuration, i.e., without the use of an annular denuder, is simpler and thus recommended for measurement of the size distribution of PAHs in either group.

Seasonal and Spatial Variations of Vapor- and Particle-Phase PAH Levels. For all communities, we found that naphthalene (NAP) accounted for 95% of the total PAH mass, with annual averages ranging from 89 to 142 ng/m^3. The highest values for benzo[ghi]perylene (BGP), a tracer of light-duty engine exhaust (present almost exclusively in the particle-phase), and of the pro-carcinogen benzo[a]pyrene (BAP) were observed in Long Beach and Lancaster. Annual averages of the pro-carcinogen BAP were also highest in Long Beach and Lancaster (Eiguren-Fernandez, et al., 2004; Eiguren-Fernandez, et al., 2007). A considerable increase in the particle-phase PAH level, relative to the vapor-phase, was observed as ambient temperature decreased. Cold/hot season ratios for PAHs in $PM_{2.5}$ reached 54 at Long Beach. These data underscore the importance of seasonal variation on PAH concentration, expressed as lower

surface and boundary inversion layers and reduced advective atmospheric mixing during the cold season, as compared with the effects of PAH chemical reaction with atmospheric gases and free-radicals during the hot season.

Seasonal Variation of the Size Distribution of PAH, EC and Major Ionic Species Downwind of Central Los Angeles. Samples were collected approximately once every week, for 24-hour periods, from midnight to midnight. MOUDI impactors samples were composited for analysis into monthly periods in three aerodynamic diameter size intervals, defined for the purpose of this report as UF, F, and C. For the monthly composites from October to February, the size distributions of the target PAHs are similar. However, from March to July, notable differences were observed: a significant fraction of the PAH mass was found in the C, as compared with the previous period. During the entire 1-year period, the form and shape of the EC size distributions did not vary much and were distinguished by prominent mass in the UF and accumulation size mode. For the individual modes of the major species, the highest Pearson's correlation coefficients (r) for the variation of temperature with species concentration were found in the UF for both SO_4^{2-} (0.92) and EC (0.90), and in the C for both OC (0.85) and NO_3^- (0.54). High SO_4^{2-} correlations are consistent with increased gas-to-particle formation during the warmer months from (precursor) SO_2 emissions in the Los Angeles and Long Beach seaport areas and, similarly for EC, increased atmospheric transport to Claremont as the season progressed from winter to summer.

Observations of PAHs in the Aitken Size Range. Several dynamometer studies reported over the last decade, and more recently, from vehicle-chase studies (David Kittelson's group in Minnesota), showed that aerosols produced by spark-ignition and diesel engines and by other high-temperature processes contain nanoparticles in the Aitken, nucleation, accumulation mode, and often, fractal-like particles or agglomerates. Fuel and combustion generated PAHs that accumulate in the engine's lube oil are normally emitted through the exhaust system. Aitken size range PAHs may result from rapid cooling (self-nucleation) while the hot vapors move along the exhaust pipe into ambient air, or, as hypothesized by McMurry (2004, personal communication) they may partition from the vapor-phase into oily droplets originated from self-nucleated lube oil. While it is still unknown at present, which mechanism(s) contribute to the observed mass in the Aitken size range, our most recent observations of the size distributions of the twelve target PAHs in the 10 nm< Dp<2.5 μm size range, show that, for all target PAHs, regardless of vapor pressure, the masses in each of the differential fractions (10–18nm, 18–32nm, and 32–56nm) are larger in the smallest fraction than that in the two larger fractions (Miguel, et al., 2005). We found that, the masses in the UF size bin ranged from 100% for benzo[k]fluoranthene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene to 46% for anthracene, of the masses collected in the Aitken size range.

Modeling Study to Quantify Population Exposure to the Emissions of Naphthalene Throughout Southern California. Our results (Lu, et al., 2005) showed that gasoline and diesel engine exhaust, with related vaporization from fuels, were found to contribute roughly half of the daily total naphthalene burden in Southern California. A more detailed account of this study is reported elsewhere in this final report.

Results

Caldecott Tunnel Sampling Campaign. As part of Year 6 of our PM Center activities, we measured $PM_{2.5}$ emission factors, separately for heavy-duty diesel (HDD) and light-duty vehicles (LDV), of volatile, semi-volatile and particle-phase ($PM_{2.5}$) PAHs, over summer (2004) and winter (2005) periods in the Caldecott tunnel, Berkeley, California. We also obtained size-resolved emission factors for semi-volatile and particle-phase PAHs down to 10 nm Dp. PAH, CO and CO_2 samples were collected in Bore 1 (mix of light duty vehicles, LDV and heavy duty diesels, HDD) and Bore 2 (LDV only) of the Caldecott tunnel. Our results (in preparation for publication) showed that total PAH levels (i.e. vapor + particle phase concentration, in ng/m^3) were similar in both bores during summer and winter periods, with NAP accounting for 90% of the total PAH mass during both seasons. In general, for PAHs with MW>178 slightly higher concentrations were found during the winter period at both bores, while higher levels of naphthalene, acenaphthene, and fluorene were observed in summer. The most significant difference between Bore 2 and Bore 1 was observed for two of the higher molecular weight PAHs (benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene; their concentrations were two times higher in the LDV-only bore. Using these results, we calculated emission factors (emfs), separately, for LDV and HDD. LDV emfs for total PAHs (vapor + particle phase) are 2 times higher in winter (2005) compared with summer (2004); HDD emfs are three times higher in winter. Emfs for PAHs with molecular weights between 178 and 252 are ca. 4 times higher for HDD while PAHs with MW>252 are practically all emitted by LDVs. Comparing PAH emfs in $PM_{2.5}$ obtained in summer 2004 with those obtained in summer 1997 (8 years latter), a higher reduction was observed for HDD as compared with LDV; average reduction emission factors of 6 and 17 were obtained for total PAHs from LDV and HDD, respectively. These results are being written up for submission to publication in the journal *Environmental Science & Technology*.

Conclusions

A major conclusion of this study is that PAHs found in the Aitken size range represent a previously unreported particle size range, adding a fourth mode to the typical PAH size distributions found in ambient air in the nucleation, accumulation and coarse size modes. In terms of health significance, this finding is important because of the increased deposition efficiency in the alveolar area of the human respiratory tract of particles in the 10–32 nm diameter range. Particles in this size range may enter cellular and subcellular walls of target eukaryotes (Li, et al., 2000, 2004).

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Project 14: Physical and Chemical Characteristics of PM in the LAB (Source Receptor Study) (R827352C014)
Investigator: Constantinos Sioutas

Objective(s) of the Research Project: This final report covers the activities undertaken by the Aerosol Laboratory of the University of Southern California, which is part of the SCPCS. The SCPCS was associated with a U.S. EPA Supersite during the years 1999-2004 and many projects benefited from joint support. The activities performed by the Aerosol Laboratory were originally proposed in three project sub areas:

1. Characterization of the airborne concentrations, size distribution and other chemical/physical elements of ambient air particulate within the LAB in relation to health studies. PM characterization in the Exposure Core at the Aerosol Laboratory supported a wide range of health studies. The findings of these health studies are reported elsewhere in this SCPCS Final Report or in reports for other funding, and include:

    a. The aged rat pilot study (in collaboration with Drs. Froines, Kleinman and Cho, reported as Project 5, R827352C005).

    b. Acute cardiopulmonary responses to concentrated coarse particulate matter in human volunteers (directed by Dr. Henry Gong, USC Keck School of Medicine, reported as Project 12, R827352C012).

    c. Animal inhalation studies using concentrated UF, F, and C PM in source and receptor sites of the LAB (Dr. Kleinman, UC Irvine, reported as Project 4, R827352C004, with detailed report available at http://www.arb.ca.gov/research/abstracts/98-316a.htm; Dr. Harkema, Michigan State University, final report on source receptor study at http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/1762/report/0)

    d. *In vitro* studies of PM chemical and cellular toxicology (Dr. Andre Nel, UCLA, reported as project 2; Drs. Arthur Cho and John Froines, UCLA, reported as Project 1, R827352C001)

2. Development of concentrators for UF, F, and C in support of animal inhalation studies, conducted at air pollution source and receptor sites of the LAB (collaborations and other relevant reports listed in a, c, d above)

3. Development of concentrators and support of studies of human exposure to concentrated PM (in collaboration with Dr. Henry Gong, USC School of Medicine, Project 12, R827352C012)

Summary of Findings: Over the course of six years of the SCPC, extensive studies by the Aerosol Laboratory have characterized PM size distribution and chemical composition at many locations around the LAB, both in the context of Center health studies and independently. Sites identified as primarily source or receptor influenced, freeway sampling sites, indoor locations,

and specific locations associated with ongoing health studies have been studied. Taken together, the many findings establish that the physical and chemical properties of ambient PM depend greatly on locations and season. In general, UF concentrations are highest at sites heavily impacted by sources such as fresh vehicular combustion emissions. More detailed findings on the size distribution of UF at various locations are reported below. Mass concentrations in the accumulation mode and C mode showed pronounced seasonal dependence. Concentrations of these larger modes tend to be lower in winter than in summer, especially at the receptor sites.

There has been some debate as to whether a 1.0 μm or a 2.5 μm cut-point provides good separation between accumulation mode particles (combustion emissions and photochemical formation and processing) and C, mechanically-generated particles (such as soil and road dust). Although previous research does suggest a relationship between C and intermodal (1–2.5 μm) PM, the sites in which these studies were conducted are not representative of all locations. Similar data for C, intermodal, and F were collected across four sites in the LAB. While some similarities exist between these results and those of comparable studies, our Supersite studies showed that intermodal PM consists of a significant portion of particles that are similar in chemical composition to PM_1 particles that are thought to cause the greatest health effects. This study was performed to shed light on the origin and chemical composition of intermodal particles between the C and F in Los Angeles, a unique city where crustal, oceanic, and anthropogenic primary and secondary sources are responsible for the high observed PM levels. Our results indicate that a PM_1 standard would not constitute an unambiguous separation of C and F in this urban air shed. (Geller, et al., 2004).

Composition studies of ambient PM were a major focus of our work. Generally, C was composed mostly of nitrate and crustal elements (iron, calcium, potassium, silicon, and aluminum). Consistent relative levels of these elements indicate a common source of soil and/or road dust. In the accumulation mode, nitrate and organic carbon were the predominant species. Higher nitrate levels were found in accumulation mode PM from receptor sites. The UF consisted of mostly carbon, especially organic carbon (OC). At source sites, levels of OC in UFs were higher in the wintertime, attributable to increased organic vapor condensation from vehicles at the lower wintertime temperatures. Conversely, at receptor sites, organic carbon levels in the UF mode peak in the summertime for two reasons: secondary organic aerosol formation by photochemical reactions is enhanced in the summer, and increased advection of polluted air masses from upwind occurs (Sardar, et al., 2005b).

Measurement of metals in daily size-fractionated ambient PM_{10} samples was conducted at source (Downey) and receptor (Riverside) sites within the LAB. The main source of crustal metals, e.g., Al, Si, K, Ca, Fe and Ti, can be attributed to the re-suspension of dust at both source and receptor sites. All the crustals were predominantly present in super micron particles. At the source site, potentially toxic metals, e.g., Pb, Sn, Ni, Cr, V, and Ba, are predominantly partitioned (70–85%, by mass) in the sub micron particles. The receptor site exhibited C distributions for almost all particle-bound metals. Fine PM metal concentrations in that site seem to be a combination of few local emissions and those transported from urban Los Angeles. The majority of metals associated with fine particles are in much lower concentrations in the receptor compared to the source site. Coarse PM metal concentration trends are governed by variations in the wind speeds in each location, whereas the diurnal trends in the fine PM metal

concentrations are found to be a function both of the prevailing meteorological conditions and their upwind sources (Singh, et al., 2002).

UF dominate the number concentrations of ambient aerosols and have been implicated in numerous studies of the health and toxicological effects of PM exposure. For this reason, UF have been a particular focus of our work. We have conducted several detailed studies of size distribution of UF (number and mass concentrations) at various sampling sites, including indoor locations, and explored the effect of season on size and composition of particles in the UF mode. This work was complemented by in vitro studies in other SCPCS labs on the chemical and cellular toxicology of our ambient UF collections. Key UF studies are summarized in the following paragraphs.

Studies conducted in Southern California showed that the size distribution of UF in source sites was generally unimodal with a mode diameter of 30–40 nm and without significant monthly variations. In contrast, the number-based particle size distributions obtained in receptor sites were bimodal, with a significant increase in accumulation mode as the season progressed from winter to summer. Afternoon periods in the warmer months are characterized by high number counts while mass and EC remain low, suggesting the formation of new particles by photochemistry. Particle mode diameters range from 30 nm up to above 100 nm, a result not seen in most other studies of particle size distributions in other urban or rural areas where mode diameters are generally less than 50 nm. Although vehicular emissions have been assumed to dominate the observed UF concentrations and size distributions our study presents evidence that day to day UF levels are also influenced by long range advection and photochemical processes. (Fine, et al., 2004b).

The very small mass of UF has posed a great challenge in determining their size-dependent chemical composition using conventional aerosol sampling technologies. Implementing two technologies in series (the USC Ultrafine Concentrator described by Kim, et al., 2001b, and the MSP NanoMOUDI) has made it possible to overcome these two problems. UF were measured in source and receptor sites during three consecutive 3-hour time intervals, i.e., morning, midday and afternoon. The results indicate a distinct mode of UF mass in the 32–56 nm size range that is most pronounced in the morning and decreases throughout the day. While the mass concentrations at the source site decrease with time, the levels measured at Riverside, CA (a "receptor" site) are highest in the afternoon with a minimum at midday. In that site, UF EC and OC concentrations were highly correlated only during the morning period, whereas these correlations collapsed later in the day. These results indicate that in this area, UF is generated by primary emissions during the morning hours, whereas secondary aerosol formation processes become more important as the day progresses (Geller, et al, 2002). Another study with the Nano-MOUDI explored the UF chemical composition at urban source sites (USC and Long Beach) and inland receptor sites (Riverside and Upland) in the LAB over three different seasons. Two week composite samples showed a distinct OC mode was observed between 18 and 56 nm in the summer, likely due to photochemical secondary organic aerosol formation. Collocated continuous measurements of particle size distributions and gaseous pollutants helped to differentiate UF sources at each site (Sardar, et el., 2005b).

It has been hypothesized that UFs originate primarily from vehicular emissions; thus, the concentrations of gases such as CO, NO, or NO_2 that also originate from traffic sources could be used as surrogate measures of UF. The advantage of this approach is that concentrations of these gases are monitored routinely in compliance networks and on personal levels by means of relatively simple and easy-to use monitors. The validity of the assumption that gases can be used as surrogate measures of UF was tested in five sites of the LAB over the course of one calendar year. Our studies indicate an overall lack of significant associations between hourly and 24-hr UF particle number and gaseous co-pollutant concentrations. The findings can be attributed to the differences in the sources and formation mechanisms that are responsible for generating these pollutants in various locations of the LAB. These findings also imply that potential confounding effects of co-pollutants will not affect epidemiologic analysis seeking to link UF to health effects because of the weak to moderate associations between PM and co-pollutant concentrations. (Sardar, et al., 2004).

In collaboration with the CARB, we developed a mobile platform designed to characterize UF and associated pollutants inside vehicles. The equipment was applied during commutes in Los Angeles freeways and residential streets. Freeway exposure concentrations were frequently an order of magnitude higher than on residential streets for UF, NOx, BC, and CO, with higher NO_x and BC values observed in diesel traffic freeways than those with mostly light duty vehicle traffic (Westerdahl, et al., 2005).

We conducted a study to evaluate contributions of vehicle generated UF to indoor environments in close proximity to freeways in the absence of known indoor aerosol sources. Indoor/outdoor (I/O) ratios of particle number concentration showed a strong dependence on particle size and were influenced by different ventilation mechanisms. Among different size ranges of UF, the highest I/O ratios (0.6–0.9) were generally observed for larger ultrafine particles (70–100 nm), while the lowest I/O ratios (0.1–0.4) occurred typically around 10–20 nm. The size distributions of indoor aerosols showed less variability than those of outdoor freeway aerosols. The penetration factors and deposition rates also varied significantly depending on particle size and agree with literature data and theories for particles greater than 20 nm. For particles less than 20 nm, I/O ratios, penetration factors, and deposition rates did not conform to theoretically predicted values. UF from freeways are unique particles with a high semi-volatile content. The sub-50 nm particles are especially high in semi volatiles and thus shrink to a smaller size (or evaporate completely) as they infiltrate indoors (Zhu, et al., 2005). These results were further supported by a study in which we examined volatility of penetrating ultrafine outdoor particles, predominantly from freeway emissions, into indoor environments using a tandem differential mobility analyzer (TDMA).

Evaluation of outdoor particle volatility as a function of distance to the freeway revealed that aerosol volatility decreases with increasing distance from the source (Kuhn, et al., 2005b). Physical and chemical characteristics, including volatility of PM in the proximity of a Light-Duty Vehicle (LDV) freeway were also measured and analyzed. The volatile component ranged from about 65% volume of 120 nm particles heated to 110°C, to 95% volume of 20 nm particles (Kuhn et al., 2005a). Our freeway PM measurements were compared to those performed in two bores of the Caldecott Tunnel in Northern California. One bore (Bore 1) is open to both heavy- and light-duty vehicles (HDV–LDV) while heavy-duty vehicles are prohibited from entering the

second bore (Bore 2). A strong association between particle number and normalized vehicle speed ($R^2 = 0.69$) was observed, and heavy-duty diesel vehicles showed higher particle number emissions than light-duty vehicles. Compared to previous studies at the Caldecott Tunnel, less particle mass but more particle numbers (by factors of 2–4 fold) are emitted by vehicles than was the case 7 years ago. As the emissions of carbonaceous PM of newer engines decreases, the formation of nucleation mode particles is favored due to the reduction of the available surface for adsorption of the semi-volatile material. The resulting supersaturation of the mostly organic vapor increases the production of nano-particles by nucleation (Geller, et al., 2005).

Another focus of our work on particle characterization concerns the development of tracer species. Individual organic compounds can be used as tracers for primary sources of ambient PM in chemical mass balance receptor models. By examining the seasonal, temporal, geographical, size-fractionation, and inter-correlations of individual organic compounds, the sources and atmospheric fate of these tracers can be better understood and their utility as molecular markers can be assessed. Investigators in the SCPCS have used a high-flow rate, low pressure-drop UF separator to collect sufficient mass for organic speciation of UF and accumulation mode aerosol on a diurnal basis. Sampling was conducted at two sites (source and receptor) over two seasons (summer and winter). Hopanes, used as organic markers for vehicular emissions were found to exist primarily in the UF. Levoglucosan, an indicator of wood combustion, was quantified in both size ranges but more was present in the accumulation mode particles. An indicator of photochemical secondary organic aerosol formation, 1,2 benzenedicarboxylic acids, was found primarily in the accumulation mode and varied with site, season and time of day as one would expect for a photochemical product. These data will be used to assess the concentration of specific PM sources to personal exposure and ultimately health effects in upcoming epidemiological and toxicological studies in LAB (Fine et al., 2004a).

C, F, and UF concentrator technologies have been developed and evaluated by investigators of the SCPCS, as well as support from the CARB. These instruments are portable and have been demonstrated to increase ambient particle levels by enrichment factors up to 40 without significantly affecting particle properties such as size (Misra, et al., 2004), bulk chemistry (Kim, et al., 2001a; Khlystov, et al., 2005) or single particle chemistry (Zhang, et al., 2004) and morphology (Kim, et al., 2001a). These concentrators have been applied in a range of studies to provide elevated ambient PM exposures to animal or human subjects, as well as to collect large quantities of PM material in aqueous solution suitable for subsequent toxicological assays. Highly concentrated liquid suspensions of these particle modes were obtained by connecting the concentrated output flow from each concentrator to a liquid impinger (BioSampler™, SKC West Inc., Fullerton, CA). Particles are injected into the BioSampler in a swirling flow pattern so that they can be collected by a combination of inertial and centrifugal forces. This inertia-based collection mechanism, coupled with the short residence time (i.e., order of 0.2 seconds) of particles and gases in the Biosampler precludes any inadvertent trapping of gaseous co-pollutants in the particulate layer. Two different particle mass spectrometers, the Aerodyne Aerosol Mass Spectrometer (AMS) and the UC Davis Rapid Single-particle Mass Spectrometer (RSMS-3), were to evaluate the performance of the Versatile Aerosol Concentration Enrichment System (VACES) developed by USC. The RSMS-3 experiments were conducted as part of the U.S. EPA Supersite program in Pittsburgh during March 2002. RSMS-3 hit rate increases were measured and possible particle composition changes introduced by the VACES were examined

in the single particle mass spectra. Both ambient and concentrated carbonaceous and ammonium nitrate composition distributions were indistinguishable with RSMS-3 suggesting that VACES introduces an insignificant artifact for those particles (Zhao, et al., 2005). The effect of concentrating semi-volatile aerosols using the VACES and the Aerodyne AMS during measurements of ambient aerosol in Pittsburgh, PA was also investigated. It was found that the shape of the sulfate mass-weighed size distribution was approximately preserved during passage through the concentrator for all the experiments performed, with a mass enhancement factor of about 10 to 20 depending on the experiment. The size distributions of organics, ammonium and nitrate were preserved on a relatively clean day (sulfate concentration around 7 ug/m^3), while during more polluted conditions the concentration of these compounds, especially nitrate, was increased at small sizes after passage through the concentrator. The amount of the extra material, however, is rather small in these experiments: between 2.4% and 7.5% of the final concentrated PM mass is due to "artifact" condensation (Khlystov, et al., 2005).

Collection of ambient particles on filters and in the biosampler, using the VACES technology, was performed concurrently with our PM physico-chemical characterization studies. Collected particles were supplied to other SCPC laboratories for *in vitro* toxicological studies, and the findings of the toxicological studies could then be interpreted in light of the physicochemical analysis results. The toxicology findings that derived from these studies are summarized elsewhere in this report. Examples include assays for redox properties of PM and studies of cellular toxicity including mitochondrial effects, in which UF were generally found to possess greater toxic properties compared to F and C. *In vivo* studies using CAPs in the vicinity of freeways were also supported in part by the SCPS. A series of studies found that CAPs exposure can exacerbate airway inflammation and allergic airway responses in a sensitized mouse model, with the responses being greater for UF, especially those in close proximity (within 50 m or less) of a freeway (Kleinman, et al., 2005; Campbell, et al., 2005). Neurological inflammation and cardiovascular effects were also observed in close proximity to freeways.

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Project 15: Exposure Assessment and Airshed Modeling Applications in Support of SCPC and CHS Projects (R827352C015)
Investigators: Richard P. Turco, Arthur M. Winer, Frederick W. Lurmann, Rong Lu, Jun Wu

Objective(s) of the Research Project: Two overall objectives of this integrated modeling project were to utilize airshed model outputs as inputs to exposure assessment models designed to quantify long-term average exposure of subjects in the CHS to criteria pollutants; and assess population exposure to ambient naphthalene concentrations in Southern California. In the course of this research we also demonstrated significant problems with the accuracy of widely-used roadway networks, and geocoded addresses. In addition, we developed methods for improvements in these important parameters, and also investigated environmental justice implications of traffic-related air pollution in Southern California.

The major elements of this final report are:

1. Exposure Assessment

    a. Exposure assessment for the CHS Children
    b. Naphthalene exposure assessment for the population of Southern California
    c. Improving spatial accuracy of roadway network and geocoded addresses
    d. Environmental justice and traffic-related pollution in Southern California

2. Airshed Modeling

    a. Regional distributions and behavior of particulate-borne trace metals in the LAB
    b. Properties of diesel exhaust in the LAB: elemental carbon sources and distributions
    c. Distributions of Naphthalene and its derivative quinones in the LAB
    d. Application of the Surface Meteorology and Ozone Generation (SMOG) model in support of the SCPC CHS
    e. Modeling UF near major roadways for human exposure assessment.

Summary of Findings:

Exposure Assessment for the CHS Children

Objectives. The overall objective of this modeling study was to provide, retrospectively, more accurate and comprehensive assessments of the long-term average exposure of the individual children in the CHS to vehicle-related pollutants. The specific objectives of the present study were to quantify the variability of within-community exposures; to determine exposures due to local mobile source emissions (LMSE) relative to meteorologically-transported pollutants and local non-mobile source emissions (LN-MSE); and to facilitate evaluation of relationships between exposure and health outcomes for the individual children in the CHS cohort.

Project Summary. We developed an Individual Exposure Model (IEM) to retrospectively estimate the long-term average exposure of the individual CHS children to vehicle-related pollutants, including CO, NO_2, PM_{10}, $PM_{2.5}$ and elemental carbon ($PM_{2.5}$ portion). Exposures were estimated by tracking children's time-activity in five microenvironments (residential outdoor, residential indoor, school outdoor, school indoor, and in vehicle) where children spend most of their time. A time-activity submodel was developed to create 24-hour time-activity series for each child in the CHS cohort by using information from both the CHS survey and the Consolidated Human Activity Database (CHAD) developed by the U.S. EPA. The CHS children were grouped by age, gender, day-type, time outdoors and time-in-vehicles. For each CHS category a child was grouped into, the corresponding CHAD distribution was sampled.

In the IEM, pollutant concentrations due to both local mobile source emissions and meteorologically-transported pollutants were taken into account by combining a line source model (CALINE4) with the SMOG model. To avoid double counting, local mobile sources were removed from SMOG and added back by CALINE4. The basis for this approach is that vehicle-related primary pollutant concentrations can be separated into, and modeled as local mobile source emissions, and transported and local non-mobile source emissions. Indoor pollutant concentrations were estimated from a single-compartment, steady-state mass balance equation, through which contributions from indoor and outdoor sources were separated. In-vehicle pollutant concentrations were estimated based on recent in-vehicle and in-bus measurements conducted in California. Pollutant exposures due to outdoor origins were estimated for seven CHS communities in the California South Coast Air Basin (SoCAB), including Lancaster (LAN), San Dimas (SDM), Upland (UPL), Mira Loma (MRL), Riverside (RIV), Long Beach (LGB), and Lake Elsinore (LKE).

The study showed that the ratio of pollutant concentrations from transport and local non-mobile sources was correlated inversely with traffic density. Most PM_{10} and $PM_{2.5}$ came from transport and local non-mobile source emissions, in agreement with recent studies showing that PM_{10} and $PM_{2.5}$ act more like regional pollutants rather than reflecting direct emissions from motor sources. Transported CO (including mobile source emissions from upwind locations) and LN-MSE comprised over 60% of the CO concentrations at all of the CHS communities except Lancaster.

Model simulations were conducted for three scenarios: with local traffic adjustment and time-activity simulation (total exposure estimated using combined local and transported pollutant concentrations); without local traffic adjustment but with time-activity simulation (total exposure estimated using air monitoring station data); and without local traffic adjustment or time-activity simulation (annual average ambient concentrations). Personal PM_{10} exposures estimated for these three scenarios are plotted in Figure 10. We found local traffic significantly increased within-community variability for exposure to CO, NO_2, and PM-associated pollutants, especially at communities with heavy traffic (e.g. Long Beach). The overall within-community variability of personal exposures (including local traffic effects and time-activity differences) were highest for NO_2 (±20–40%), followed by EC (±17–27%), PM_{10} (± 15–25%), $PM_{2.5}$ (±15–20%), and CO (±9–14%), where the ranges are across seven CHS communities. Local traffic alone contributed most to CO and NO_2 exposures, but less to PM-related exposures. Significant within-community variability due to children's time-activity patterns was also observed, with the highest for NO_2 (±18–25%), followed by EC (±16–20%), PM_{10} (± 12–18%), $PM_{2.5}$ (±10–15%), and CO (±3–9%).

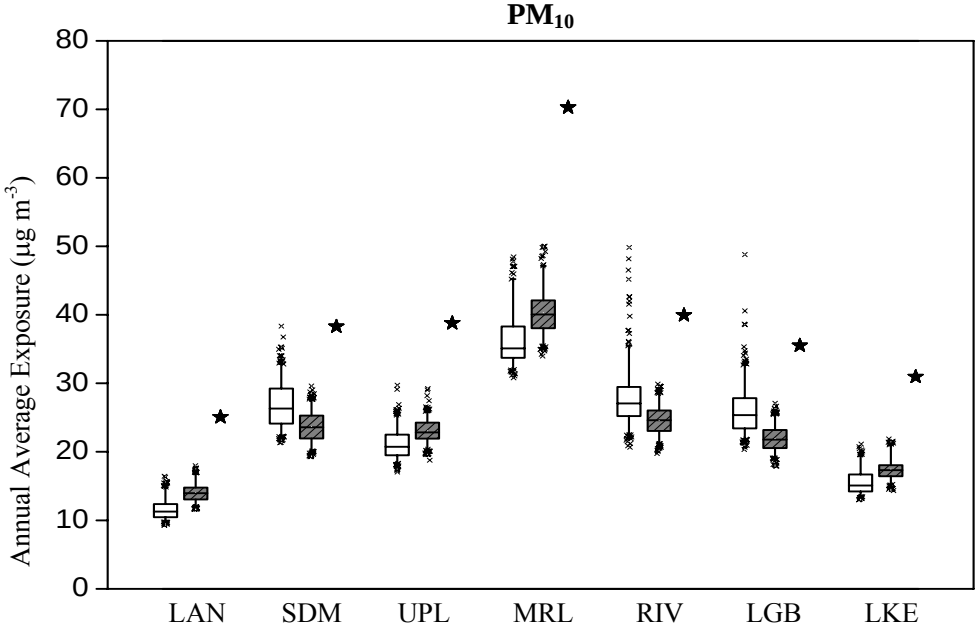

Figure 10. Estimated Annual Average Exposures of the CHS Children in 1997 Under Three Scenarios

Total exposure calculated using combined local and transported pollutant concentrations
Total Exposure calculated using monitoring station data; annual average ambient concentrations

Differences in community mean exposures were impacted by community location (e.g. source vs. receptor areas), traffic density, locations of residences and schools within a community. For CO, the community mean personal exposure ranked RIV (1.8) > LGB (1.7) > SDM (1.5) > MRL (1.0) > LAN (0.7) (community mean exposure in mg m^{-3}). For PM_{10}, the community mean exposure ranked MRL (36.4) > RIV (28.0) > SDM (26.8) > LGB (26.1) > UPL (21.1) > LKE (15.7) > LAN (11.6) (in μg m^{-3}).

Conclusion. Local traffic significantly increased within-community variability for exposure to CO, NO_2, and PM-associated pollutants, especially in communities with heavy traffic. Exposures to PM-associated pollutants were impacted more by transport and local non-mobile source emissions. Significant within-community variability due to time-activity pattern differences was observed. Between-community exposure differences were affected by community location, traffic density, locations of residences and schools, and time-activity patterns of the children in each community. The ambient pollutant concentrations measured by the CHS central monitoring stations did a reasonable job of capturing the range of residence and school outdoor concentrations at Lancaster, Lake Elsinore, Upland, and Mira Loma, but not at San Dimas, Riverside, and Long Beach. This monitoring station "siting" issue has implications for epidemiological studies since many of these studies use ambient concentrations as surrogates for personal exposures.

Naphthalene Exposure Assessment for the Population of Southern California

Objectives. Naphthalene, a hazardous air pollutant and a potential carcinogen, can irritate the eyes, skin and respiratory tract, cause kidney and liver damage, and attack the central nervous system (USEPA, 2003). No exposure assessment has been conducted for the present SoCAB population due to extremely limited measurement data. The objective of this study was to estimate naphthalene exposure for the SoCAB population by applying a sophisticated air quality model and a human exposure model.

Project Summary. We linked the SMOG airshed model and the Regional Human Exposure (REHEX) model to assess human exposure to air pollutants in the SoCAB. Hourly ambient naphthalene concentrations predicted by the SMOG model were fed into the REHEX model as outdoor pollutant concentrations at a 5-kilometer resolution. Ratios of indoor-to-outdoor (I/O) naphthalene concentrations were obtained from the Fresno Asthmatic Children's Environment Study (FACES) in California, in which 150 naphthalene samples were collected inside and outside 47 non-smoker homes from 2002 to 2003. In the absence of any in-vehicle naphthalene measurements for the SoCAB, an average in-vehicle-to-ambient naphthalene ratio of three was assumed for this exposure assessment. Time-activity patterns for California residents were obtained from the CHAD.

Results showed mean hourly naphthalene exposures of 270 ng m^{-3} and 430 ng m^{-3} for the SoCAB population in the summer and winter episodes, respectively. These mean hourly exposures were

about 80% higher than the population-weighted outdoor concentrations (150 ng m^{-3} and 230 ng m^{-3}) because of indoor sources and in-vehicle exposures. More than one million people in the SoCAB were exposed to naphthalene at mean concentrations exceeding 1000 ng m^{-3} during the winter period. Remarkably, about 65,000 and 1,300 people experienced naphthalene exposures exceeding 2000 ng m^{-3} and 3000 ng m^{-3} respectively, in the winter case.

Population naphthalene exposures correlated strongly with transportation corridors in the SoCAB since gasoline and diesel products and motor vehicle exhaust contribute more than half of the total emissions in the basin. High naphthalene exposures appeared in areas where both emissions and population densities were great, such as the downtown Los Angeles area. In addition, naphthalene exposures showed a high degree of regional-scale variability, from less than 50 ng m^{-3} to up to 1300 ng m^{-3} in 5-km grids, indicating that more than single-point sampling is required to characterize properly naphthalene and other PAHs in an urbanized region.

Indoor sources and travel by vehicles accounted for 40% and 4% of the total exposure, respectively. Exposure due to environmental tobacco smoke (ETS) was 5–14 ng m^{-3}, which accounted for only 1–5% of the total exposures, indicating that ETS is not a significant source of population naphthalene exposure, at least for the SoCAB population where smoking rates have declined significantly in recent years. However, we did not calculate naphthalene exposures due to direct smoking activities of single individual smokers, whose exposure to naphthalene while smoking would be significantly higher.

Conclusion. By linking the SMOG and REHEX models, we obtained the first comprehensive quantitative assessment of the population exposure to naphthalene in the SoCAB. Average hourly naphthalene exposures in the SoCAB under summer and winter conditions were 270 ng m^{-3} and 430 ng m^{-3}, respectively. Certain individuals experience much higher concentrations of naphthalene. More than one million and one thousand individuals were estimated to experience naphthalene exposure greater than 1000 and 3000 ng m^{-3}, respectively. Substantial spatial and temporal variations exist for naphthalene exposures, with populations living, working, or attending school adjacent to major roadways experiencing the highest exposures. Indoor sources and travel by vehicles accounted for 40% and 4% of the total exposure, respectively, while ETS accounted for less than 5% of total naphthalene exposures. The highest naphthalene exposures estimated from this modeling study exceed the reference concentration for chronic inhalation exposure adapted by the U.S. EPA (3000 ng m^{-3}).

Improving Spatial Accuracy of Roadway Networks and Geocoded Addresses

Objectives. Recent field studies indicate the highest exposures to direct vehicle-related pollutants are highly localized (within a few hundred meters) near major roadways (Hitchins, et al., 2000; Zhu, et al., 2002a; 2002b), suggesting a high spatial resolution, down to tens of meters, may be required to better characterize exposure to motor vehicle-related pollutants. Two main concerns arise when conducting exposure assessment studies on vehicle-related pollutants: 1) accurately mapping (geocoding) the residence locations of study participants; and 2) accurately mapping and characterizing road networks and traffic activity. The objectives of this study were to develop GIS-based methods to improve the accuracy of roadway network data associated with

traffic activities; provide the optimum geocoding data available; assess the magnitude of potential exposure errors associated with spatially inaccurate roadways and misplaced addresses; and provide refined exposure assessment of vehicle-related pollutants to support the CHS.

Project Summary. We obtained vehicle activity data for southern California from the California Department of Transportation (Caltrans). The TeleAtlas MultiNet™ USA (TAMN) database was also obtained since it had detailed roadway and address attribute information, a high degree of positional accuracy, and geocoding capability. The Caltrans roadways were based on United States Geological Survey (USGS) data with traffic activity data; while the TAMN roadways were based on global positioning system (GPS) validation but lacking traffic information.

Results showed that in highly urbanized areas there was generally good agreement between the spatial positioning of the Caltrans road network and the TAMN data, however, for some urban areas and several suburban areas, differences on the order of 50 m to 400 m were identified between the two datasets (Figure 11). In Los Angeles County, average discrepancies between the matched Caltrans and TAMN roadway segments were 21 m, 30 m, and 32 m for freeways, major roadways, and minor roadways, respectively. However, the actual difference between unmatched roadway segments could be much larger and more difficult to quantify.

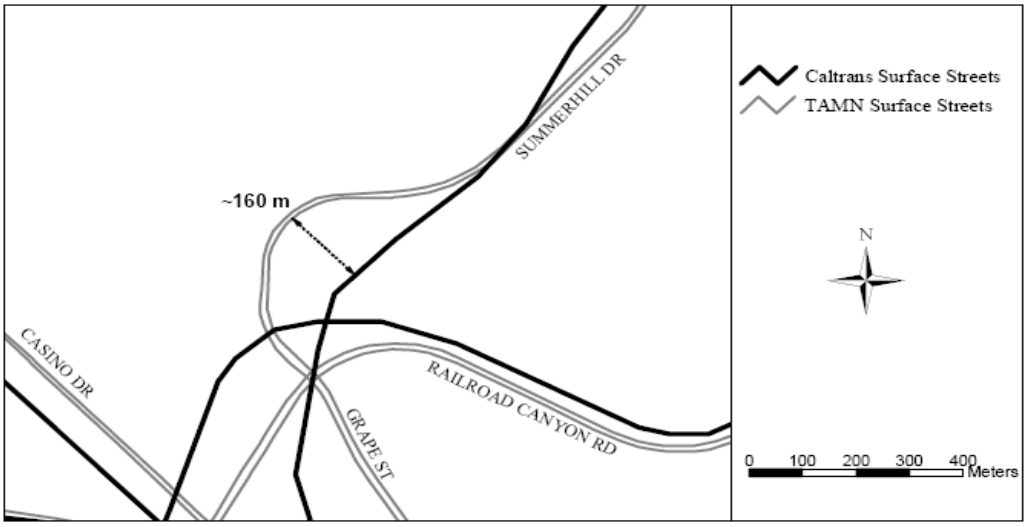

Figure 11. Comparison Between Caltrans and TAMN Roadway Network for Surface Streets in Riverside, CA, Showing Discrepancies Up to ~160 m

TeleAtlas geocoding service was used to locate 8,593 addresses. Ninety-seven percent of addresses were matched to an exact house number to the correct side of the street or unique intersection. Two percent of the addresses were located to the correct block but with unknown position along the block, which occurred most often in the rural communities of Lake Arrowhead, Alpine, and Lompoc or when an address was part of a large apartment complex. These addresses were manually corrected in 90% of cases, using aerial photography from the USGS Terraserver, MapQuest, Yahoo, and the USPS Zip4 website. The average interpolation error in geocoding can be characterized as ± one-half the length of a block, which corresponds to

75–140 m for large arterial and local roads in the urban areas, and 100–200 m for roads in the rural areas, respectively.

A GIS-based processing scheme was developed to transfer the Caltrans vehicle activity information onto the TAMN road network database. An automated GIS algorithm was developed, which outputs a relational cross-reference file that matches Caltrans road links (with vehicle activity and fleet composition) to the corresponding TAMN road segments. The transferring of the Caltrans annual average daily traffic (AADT) counts to the TAMN database yielded a match rate exceeding 95%. The unmatched pairs were mostly due to name mismatches (e.g. misspelling in the names) or large discrepancies in the geometry of roadway links (e.g. a link shown in Caltrans roadway network might not exist in the TAMN network). Manual matching was applied to facilitate assigning the unmatched Caltrans links to the TAMN data, especially in cases where street names were misspelled.

CALINE4 model sensitivity runs were conducted under different meteorology conditions and receptor locations. For a simple case of light daytime winds (2 m/s, with slightly unstable atmospheric conditions) blowing perpendicular to a busy, at-grade freeway (10,000 vehicles per hour), the CALINE4 model estimated a factor of eight difference in the concentrations of carbon monoxide at receptors located 30 m and 300 m downwind of the roadway centerline. Using a single line to represent a freeway (in Caltrans data) or double lines for two directions of a freeway (in TAMN data) can also result in different pollutant concentration estimates. The CALINE4 sensitivity test showed that a two-direction freeway scenario resulted in pollutant concentrations 30% lower than a single freeway scenario at a receptor 30 m downwind of the freeway.

Conclusion. This study demonstrated that large discrepancies, up to hundreds of meters, may exist among different roadway network data. Relative misplacements of roadways and participant locations of this magnitude can lead to significant misclassification of exposure estimates, up to an order of magnitude in vehicle-exhaust pollutant concentrations. Studies concerned with human exposure to air pollutants adjacent to roadways must account for such discrepancies. We successfully developed and applied a GIS method to address this problem (Wu, et al., 2005b).

Environmental Justice and Traffic-Related Air Pollution in Southern California

Objectives. Health studies showed that residents of inner-city, minority, or low-income areas were at higher risk for chronic respiratory disease because of air pollution (Eggleston, et al., 1999; Mann, 2000; Weiss, et al., 1992). Documenting and quantifying the distribution of traffic density in southern California is an essential step and prerequisite to understanding potential exposure patterns and in formulating policy and planning interventions that can help minimize the hazardous impact of vehicle-related pollutants for all residents. The objectives of this research were to identify the geographic pattern of race and poverty in southern California; document the overlap of disadvantaged neighborhoods and the transportation network; and quantify the potential disparities in traffic volume by neighborhood type.

Project Summary. Race and ethnic population data were obtained from Census 2000. The distribution of parcels by residential type (single family and multifamily) was derived from the Statewide Database of the University of California at Berkeley. Vehicle activity data for southern California were obtained from the California Department of Transportation. Neighborhood measures and traffic volume for block groups were aggregated in five southern California counties (Los Angeles, Orange, Riverside, Ventura, and San Bernardino), and by two race-based classifications (Minority and Non-Minority) and four poverty-based classifications (Very Poor, Poor, Low Poverty, and Not Poor). Block groups with over 40% of residents in poverty were classified as "Very Poor".

Based on 2000 census data, the Los Angeles–Long Beach Metropolitan Statistical Area had a Dissimilarity Index score of 67 for African Americans, indicating that that 67% of African Americans in that metropolitan area would have to move in order to be evenly distributed among Non-Hispanic Whites in the region. Patterns of racial segregation were closely intertwined with the persistence of concentrated poverty in southern California. Between 73–86% of poor residents would have to relocate within the region in order to be evenly distributed among the non-poor in the region. Although certain neighborhoods with predominately minority populations were in outlying areas, most were located in the densely populated urban core of the region, particularly in areas near downtown Los Angeles and South Los Angeles.

The separation of residents based on race and income functionally divided residents spatially based on a number of related socioeconomic characteristics. Residents of minority and very poor had lower levels of educational attainment. The very poor areas were 92 percent Non-White, and 61 percent of residents had less than a high school education. These areas also had lower rates of labor force participation. Whereas 81 percent of working age residents of non-minority neighborhoods was in the labor force, only 68 percent of working age residents of

minority areas participated in the labor force. This rate was even lower in Very Poor neighborhoods. Minority and Poor areas had a higher population density and a higher prevalence of multifamily residential parcels and older buildings than the remainder of the region. Whereas only 18% of residential parcels in minority areas were multifamily, 32% and 48% were multifamily in Poor and Very Poor areas, respectively. Residents of disadvantaged areas also had fewer transportation resources. Whereas approximately 95% of households in non-minority areas have a household vehicle, only 86% in minority areas had a vehicle. Eighty-one percent or fewer had access to a vehicle in poor and very poor areas, compared to 96% in not-poor neighborhoods. Furthermore, a lower percentage of workers traveled to work by auto in disadvantaged neighborhoods while a higher percentage traveled by public transportation.

We found that the road density of very poor areas was almost two times that of the least poor neighborhoods. Minority areas have almost 2.5 times the traffic density of non-minority areas. Very Poor areas, which represent the most disadvantaged areas in the region, had a significantly higher traffic density than minority or poor areas, perhaps because these areas have the highest roadway density. Given the magnitude of the disparities in the distribution of traffic by these neighborhoods, there is reason to suspect that residents of minority and poor areas are at a higher risk of the health effects associated with vehicle-related pollutants.

Almost half (44%–47%) of vehicle miles traveled (VMT) in the study region were on major roads as compared to highways, indicating that the adverse effects of traffic density are spread across the urban roadway system and not confined to highways. The distinction of traffic levels between major roads and highways was even more pronounced for high-density areas. While less than 40% of VMT was on major roads in the lowest density areas, 56% of VMT was on major roads in areas of the region's highest density. Clearly, non-highway arterials played a major role in sub-regional traffic patterns and should be included in future research.
Conclusion. Disparities in local traffic patterns in southern California were embedded within regional social and economic forces. Minority and high-poverty neighborhoods were distinct from other areas in the region. In aggregate, they had a higher population density, lower housing values and a high density of highways and major roads. These neighborhoods bear over two times the level of traffic density compared to rest of the region. Furthermore, they have older and more multifamily housing, which is associated with higher air exchange rates or higher indoor exposure to outdoor pollutants.

Airshed Modeling

Objective. Modeling the distributions of particle-borne materials and gaseous pollutants across the LAB is crucial for assessing the health impacts of emissions on the regional population of the LAB. It is especially important for sparsely measured, but critically important, components of smog such as elemental carbon, polycyclic hydrocarbons and their photochemical byproducts, trace metals, peroxides, and other suspicious substances. Results from model simulations are useful for characterizing the spatial and temporal variations and sources of agents of interest to the SCPCS health research team, and therefore contribute to the regulatory role of the EPA and other agencies.

The principal objective of the airshed modeling was to provide modeling support for several of the projects in the SCPCS, including the exposure assessments for the CHS. The SMOG airshed model was used to determine background pollutant abundances used in detailed CALINE4 model calculations, yielding more accurate and comprehensive evaluations of long-term average exposures of the individual CHS children to vehicle-related pollutants.

A second objective was to establish the regional distributions of pollutants for population exposure assessments with the REHEX code. Toward this end, a focused LAB scale assessment of naphthalene and its photochemical byproducts, the naphthoquinones, was undertaken. It turns out that there has been remarkably little work done on these compounds. Importantly, naphthalene is presently receiving greater attention due to its potential carcinogenic nature.

UF are suspected as causative agents for a wide range of adverse health outcomes including morbidity and mortality. Traffic from freeways and local roadways contribute to UF concentrations in adjacent communities. We have also initiated detailed modeling of UF size distributions for freeway exposure studies.

Project Summary and Accomplishments. Progress was made in a number of areas, including the modeling of distributions of particle-borne materials and gaseous pollutants across the LAB, the assessment of the regional distributions of and human exposure to naphthalene and naphthoquinones in the LAB, application of the SMOG model in support of the CHS, and development of a detailed model for UF size distributions for freeway exposure studies.

The Surface Meteorology and Ozone Generation (SMOG) Airshed Model. Over the past decade, we have developed a model that can be used to predict the distributions of gaseous and particle-borne pollutants across the LAB (Figure 12). Previous work has established the capabilities of the SMOG model through comparisons of model simulations against observations (e.g., Jacobson, 1997; Jacobson, et al., 1996; Lu and Turco, 1996, 1997; Lu, et al., 1997a and 1997b; Lu, et al., 2003; Lu, et al., 2005). The components of the SMOG modeling system are shown schematically in Figure 12 and are discussed in detail in the references provided. The specific information required for the naphthalene simulations is discussed below.

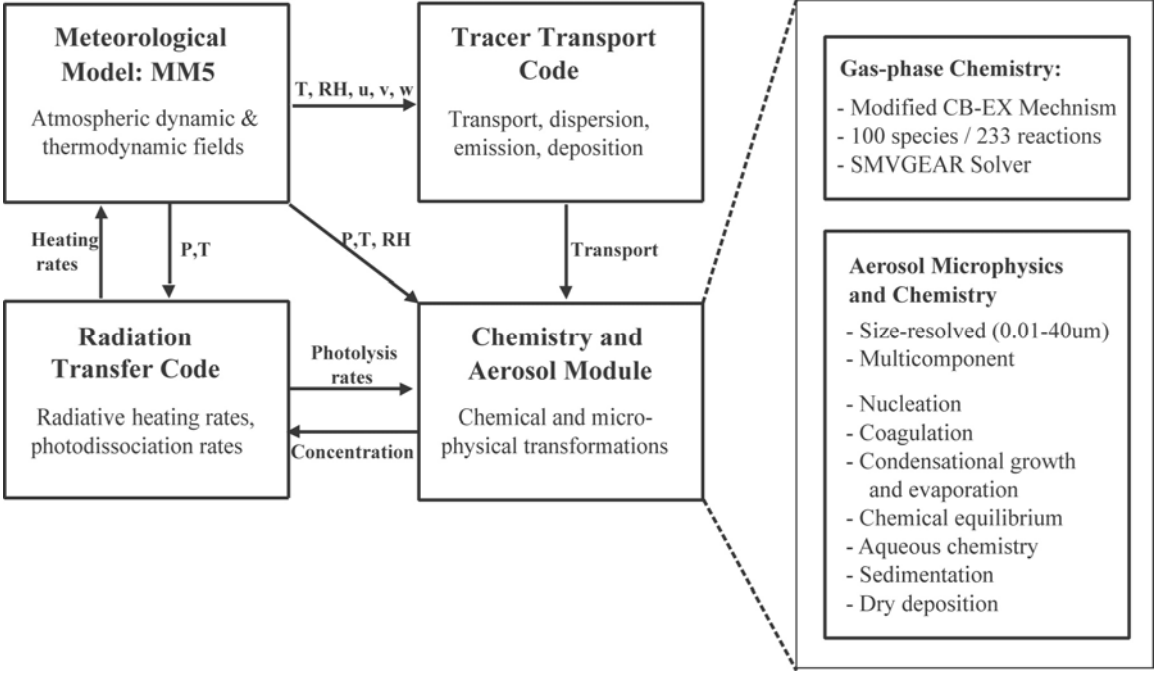

Figure 12. The SMOG Modeling System. The meteorological predictions are currently carried out using a triple-nested version of the mesoscale model (MM5) with initialization based on National Center for Environmental Prediction global data.

The regional primary pollutant emissions fluxes, photochemical reaction coefficients, and dispersion rates have been reported previously (Jacobson et al., 1996; Lu et al., 1997a). In assessing the fidelity of the SMOG model, the largest uncertainties arise from errors in the emission rates and patterns that are available from standard sources. Refinements to mobile emissions (e.g., for reactive hydrocarbons) based on suggestions from literature has been incorporated into simulations, as described and evaluated by Lu et al. (1997a; 1997b). In this study, the AQMD 1998 emission inventory has been used. The result is overall statistical agreement between model predictions and monitoring data for criteria pollutants in the range of +25–35%, with larger variability for particulates in general. In addition to emission refinements, we can also perform inverse modeling to calibrate total emissions in cases where the sources are inadequately identified or quantified. This approach is described elsewhere (Lu et al., 2003). Calibration of simulations against observations is effective in limiting biases in the predictions.

Results suggest that, in estimating human exposure to airborne pollutants, and setting standards for exposure, crucial information can be provided by regional modeling that would otherwise be difficult to obtain using ground-based measurements alone. At the same time, the distributions of co-pollutants can be obtained in a self-consistent manner.

Regional Distributions and Behavior of Particulate-Borne Trace Metals in the LAB

Trace Metal Distributions. In preliminary work to establish the capabilities of the SMOG model in simulating airborne particles and their composition throughout the LAB, we carried out the first detailed analysis of aerosol trace metals in the region, including sources, microphysical processing, meteorological dispersion, sedimentation and deposition, and ventilation from the LAB (Lu et al., 2003).

Figure 13 illustrates the predicted surface concentrations of zinc in total suspended particulates (TSP) over the LAB on a typical summer day corresponding to a recent sampling campaign. The distributions of trace metals (more than a dozen are available for analysis) are found to exhibit characteristic patterns across the LAB, which are similar to the pattern for PM_{10}. The model predictions have been checked by comparison to simultaneous PM filter samples at multiple sites (Multiple Air Toxics Exposure Study, MATES II). However, there are significant variations between the patterns for different metals. Importantly, different metals are found to be concentrated in different particle size ranges (Zn tends to lie within the larger particle fraction, for example). Our work demonstrates that the SMOG model reproduces these key properties of the trace metal distributions.

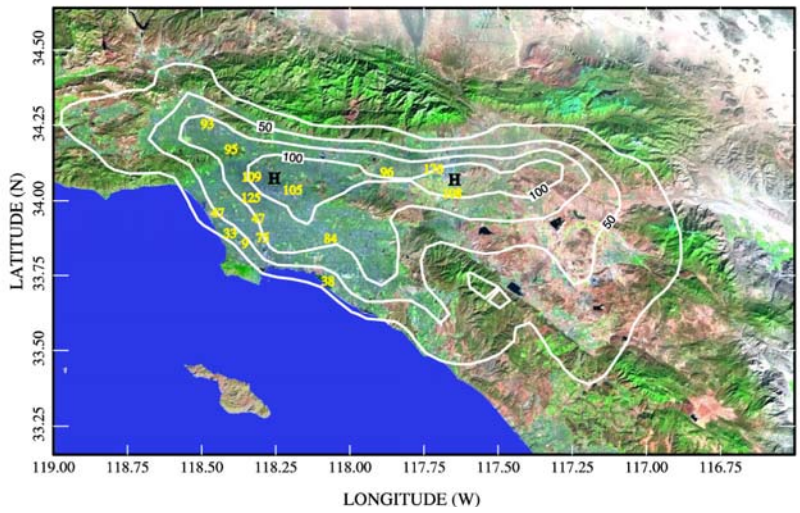

Figure 13. Comparison of SMOG-Predicted Zn Abundances (Contours) With Site Measurements (Yellow Numbers) in Nanograms per Cubic Meter (ng/m^3)
The contours represent 24-hour average total particulate Zn concentrations (at contour interval of 25 ng/m^3). The simulation corresponds to sampling periods during the MATES II project. The measured TSP (Zn concentrations) shown here are average values for the period July 1 to August 31, 1998.

Figure 14 shows the dramatic variations in the distributions of (and ultimately, exposure to) airborne particulates for different synoptic meteorological states characteristic of the LAB. Under Santa Ana wind conditions, for example, air is transported toward the southwest from the

basin, forming a pool over the adjacent ocean surface. The particulate is deposited on the surface, or is advected to other coastal regions. Clearly, exposure to aerosols in the LAB is strongly influenced by meteorology as well as proximity to sources. These new results will allow us to combine tracer distributions corresponding to various synoptic states, using the statistical frequency of occurrence, to obtain more representative estimates of mean exposure, and to quantify more realistically extreme community-wide exposures encountered in the LAB.

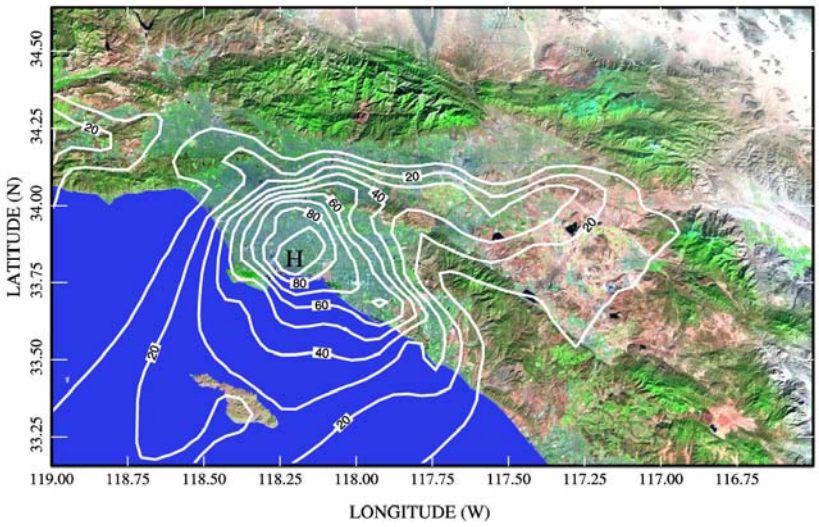

Figure 14. SMOG-Predictions of the Pb Distributions (Contours Give the Lead Abundance in ng/m^3) Across the LAB on a Day with Santa Ana Wind Conditions
The Pb contours extend over adjacent coastal waters in this case. The highest exposures would be expected to occur in the western areas of the LAB.

Results such as those in Figures 13 and 14 suggest that, in establishing exposure standards, crucial information can be provided by a regional model that would otherwise be difficult to obtain using ground-based measurements alone. Likewise, synergy between simulation and observation exists in the essential need for data to calibrate models (see below), and to define variability associated with local sources. Regional scale predictions complement site measurements by offering greater time resolution, and explicitly defining diurnal variations in surface concentrations of PM components (whereas 24-hr averaging is more typical in measuring particulates). Thus, for example, exposure at different times of the day can be reasonably estimated using modeled pollutant behavior. Finally, the distributions of co-pollutants are also available from simulations. The analysis for zinc and lead shown in Figures 13 and 14 has been extended to other aerosol trace metals, and is now available for different seasons and synoptic conditions (Lu, et al., 2003).

Calibrating Regional Simulations. We have evaluated and calibrated the emissions of particulate-borne trace metals using model simulations contrasted against regional monitoring data. In the past, this approach has been applied to calibrate volatile organic compound (VOC) emissions (Lu, et al., 1997a; 1997b). The burden of a particulate trace metal is determined as a balance between its integrated area-wide emission, subsequent meteorological dispersion, and eventual removal by deposition and ventilation. Figure 15 shows correlations of predicted and measured surface concentrations of zinc at 15 sampling sites during July and August 1998. In the SMOG calculations, we initially used emissions quantified by CARB in 1998. This led to a mean bias in the predictions that was subsequently used to calibrate—in a regional average sense—the local community-scale emissions (Lu, et al., 2003). The calibration factor is calculated as the normalized bias between predictions and observations. This analysis reveals that a single adjustment to the emissions on a regional scale is capable of bringing simulations and observations into excellent accord—with less than 20% residual mean variance (at least for the sites where data were available, and for a sample averaging time of 24 hours).

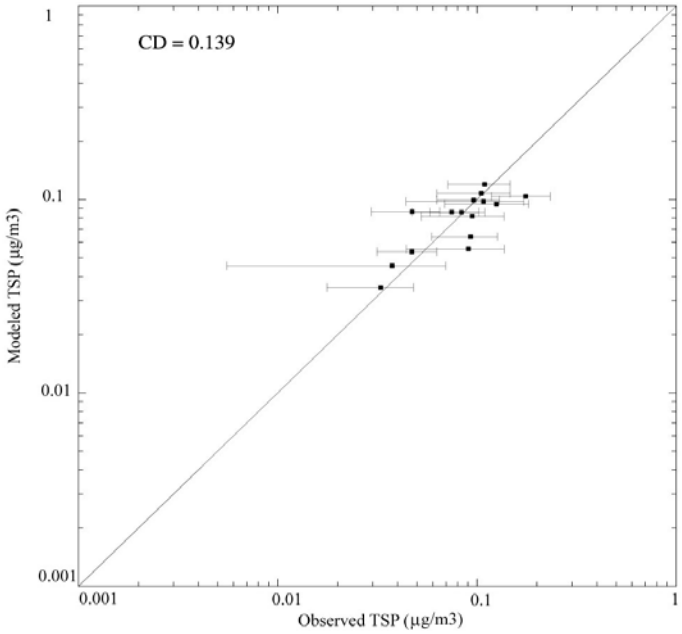

Figure 15.

Properties of Diesel Exhaust in the LAB: Elemental Carbon Sources and Distributions

The SMOG model was used to calculate and analyze the spatial distribution, size dispersion, and temporal behavior of elemental carbon (EC) in the LAB. Emission estimates for EC are available from the AQMD and CARB. We also have access to emission data on smoke aerosol components from firewood, cooking and other activities as previously compiled by center

investigator Glen Cass and his collaborators. A similar approach to that used to calibrate trace metal emissions can be applied to constrain the sources of other essentially inert materials, such as elemental carbon. The overall EC emissions in the LAB have never been tested in this way. Rather, many individual sources have been identified, and each has been quantified independently. This is where the PIU and other SCPCS observations are proving to be highly supportive of the modeling effort as we construct a more accurate regional emissions database for use in exposure assessments.

EC is treated as an unreactive tracer on the time scales of interest—that is, during dispersion and deposition throughout the LAB. A typical simulation of the EC distribution in the basin is given in Figure 16. The average distributions are obviously strongly influenced by sources along freeway corridors, noticeably along the 10 freeway in the northern and eastern basin, the 5/405 convergence in the northern San Fernando Valley, and along the Alameda corridor. The corresponding calculated mean size distribution of the elemental carbon component of the particulate shows peak abundances (by mass loading) in the UF below about one μm diameter, and in the large particle mode near 10 μm. The former peak is clearly associated with diesel exhaust, while the latter is an artifact of tire wear and road dust resuspension, and is more concentrated near freeways (note that tire rubber is treated as EC in the standard inventories).

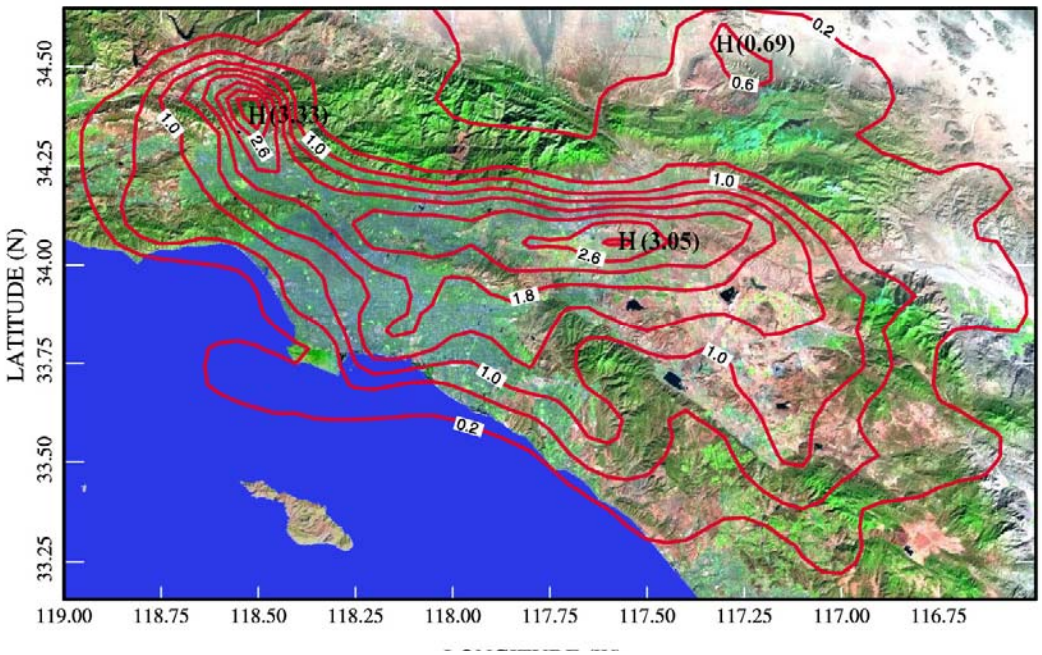

Figure 16. Distribution of Elemental (Black) Carbon, or Soot, Predicted for a Typical Summer Day

The abundances (in micrograms per cubic meter, μg/m^3) shown represent 24-hour averages. The predicted concentrations are consistent with EC data collected by the AQM

The SMOG model can be employed to resolve explicitly the temporal, spatial, and size variations associated with each distinct source of elemental carbon (e.g., diesel vehicles, stationary sources, tires, and so on). This offers the SCPCS a comprehensive characterization of such particulates for health impact assessment. Importantly, the implications of changes in specific sources can be quantified once the SMOG model is fully calibrated against SCPCS and other field measurements.

Distributions of Naphthalene and Its Derivative Quinones in the LAB

Naphthalene is the simplest and most abundant of the polycyclic aromatic hydrocarbons (PAHs), organic compounds with at least two aromatic rings fused together (Arey, et al., 1989). Although PAHs with four rings or greater are found predominately in the particulate phase, as a double-ringed PAH, naphthalene in air occurs primarily in the gas-phase and has been detected in both outdoor and indoor samples. Naphthoquinones are photooxidation byproducts of naphthalene (Atkinson, et al., 1987; Sasaki et al., 1997). The potential health effects of exposure to quinones are a current focus of research. Quinones express their toxicity through both electrophilic addition and oxidation-reduction reactions. One equivalent of quinone can generate multiple equivalents of reactive oxygen species and thus overwhelm the protective effects of antioxidant enzymes and other reducing agents (Nel, et al., 2001; Li, et al., 2000).

Naphthalene is a blood toxicant. Exposure to high concentrations of naphthalene can damage or destroy red blood cells, causing hemolytic anemia (USEPA, 2002). Naphthalene fumes can irritate eyes, skin and the respiratory tract. If inhaled over a long period of time, naphthalene may cause kidney and liver damage, skin allergy and dermatitis, cataracts and retinal damage, as well as attack the central nervous system. In animal tests, naphthalene has caused cancer as a result of inhalation (USEPA, 2002).

The regional distributions of PAHs such as naphthalene, and the formation of secondary organic compounds like the quinones and nitro-PAHs, are largely uncharacterized in the LAB. It is becoming apparent that naphthalene and its byproducts represent potential human health hazards. Naphthalene is rarely measured except in local sampling projects, and usually for limited periods. Here, the SMOG model is employed to depict the distributions of these species in much more detail than can be inferred from existing measurements alone.

Naphthalene Photochemistry. Using SMOG, we calculate the ambient abundances of these compounds based on the simplified photochemical scheme in Figure 17. The concentration of naphthalene is controlled by its reactions with the hydroxyl radical, OH, and with the nitrate radical, NO_3. Of these two reaction pathways, the OH process is more important. The concentrations of naphthalene and its immediate byproducts are largely controlled by reactions with the hydroxyl radical. The key reaction in the decomposition of naphthalene involves OH addition, for which the rate constant is known ($\sim 2.4 \times 10^{-11}$ cm^3/s at 298 K). Naphthalene also reacts with nitrate radicals, although this path is most effective at night. In the presence of NO, the yield of naphthoquinones and related species via the OH reaction can be as large as ~40%, depending on the number of compounds counted in this reactive cohort. The direct yield of 1,4-naphthoquinone is roughly 1%. Atkinson and Arey (private communication, 2001). A roadmap for the secondary reactions of several of the naphthalene products with OH has been constructed

using specific laboratory measurements as well as analogous organic chemical processes. As a result, the distributions of naphthalene and its principal secondary quinone-like products can be reasonably estimated.

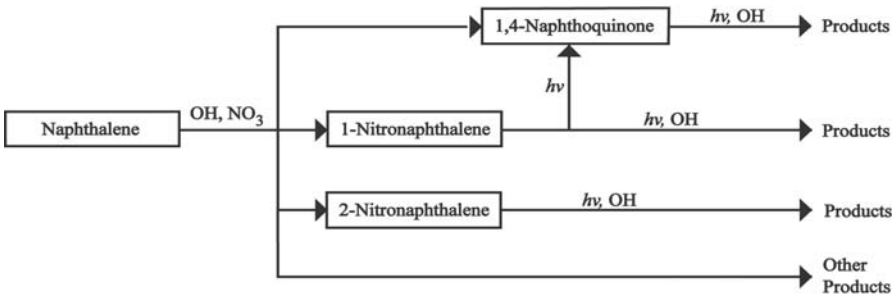

Figure 17. The Photochemical Oxidation Pathways Used to Determine the Ambient Abundances of Naphthalene and Its Quinone-Like Reaction Products

The balance for naphthalene is maintained between emissions (and dispersion) and reactions (mainly with OH). Thus, it is a simple matter to keep track of the naphthalene burden using an airshed model with a detailed photochemical mechanism (e.g., Lu, et al., 1997b). The decomposition reactions represent the sources of the quinones. The loss rates of the naphthoquinones are also dominated by OH. Hence, these products can also be tracked explicitly using SMOG simulations.

We note that naphthalene and its immediate photochemical products exist mainly in the vapor phase. Nevertheless, the product naphthoquinone and nitroquinones have lower vapor pressures and are partially partitioned onto particles. The SMOG model determines the total quantity of each compound in air, including the vapor and aerosols phases. To carry out a more thorough assessment for naphthalene-related organic compounds, physicochemical properties such as vapor pressures and solubilities must be incorporated into the modeling. For naphthalene and many other common PAHs, and their stable photochemical products, relevant data are available to determine vapor/particle partitioning factors.

Validation of Naphthalene Emission Rates. Uncertainties exist in mobile source emission factors for naphthalene, in part due to sampling difficulties. For example, naphthalene can be lost to

walls when exhaust gases are collected and stored in bags. Since naphthalene is too volatile for complete collection on a denuder, it is also necessary to back up denuders with PUF cartridges. In addition, mobile source emission factors are usually determined by sampling exhaust from a small number of vehicles under conditions that may not be representative of vehicles in use.

In contrast, benzene can be reliably measured in both vehicle exhaust and ambient air, and the ratio of naphthalene to benzene may be used to assess the reliability of naphthalene emission factors. Hence, to verify mobile source emission factors for naphthalene, we conducted a sampling experiment at the Sepulveda tunnel near the Los Angeles International Airport (LAX) with high traffic volume. The overall average naphthalene-to-benzene mass ratio determined for the tunnel samples is identical to the ratio corresponding to averaged gasoline vehicle emissions based on the CARB emissions inventory. Accordingly, the tunnel results provide strong support for the naphthalene emission rates used in the air quality simulations.

To further verify emission factors using the ratio technique, ambient air samples were collected at the California Institute of Technology, Pasadena CA, roughly 20 km northeast of downtown Los Angeles. This area is subject to light and heavy-duty vehicle traffic, as well as point and area sources of naphthalene. The naphthalene-to-benzene mass ratios for the 24-hour samples are consistent with the naphthalene-to-benzene mass ratio predicted by the SMOG model. Since benzene is emitted mainly from mobile sources, the agreement between the modeled and measured naphthalene-to-benzene ratios indicates the relative contributions to the total naphthalene emissions from mobile sources together with other sources are represented reasonably well in the emission inventory employed here.

Naphthalene Distributions Across Southern California. Figure 18 illustrates the predicted summer concentrations of naphthalene across Southern California. The regional character of the naphthalene distribution is clearly revealed with high concentrations confined largely within the basin defined by mountain ranges to the north and east and coastal ocean to the west and south. Large naphthalene gradients occur along the coast owing to prevailing winds, which transport polluted air masses inland. The peak naphthalene concentrations appear in areas where emissions are greatest; for example, adjacent to downtown Los Angeles. Winter concentrations, on average, exceed those in summer.

Naphthalene emissions are associated with fuel combustion and vaporization. Accordingly, the predicted distributions correlate strongly with transportation corridors in Southern California. For example, high concentrations stretch along the San Gabriel and San Bernardino Mountains in the northern and eastern basin corresponding to the 10 and 210 freeway corridors. Another band of high concentrations extends southeast between the San Fernando Valley and Orange County paralleling the 5 and 405 freeways. Atmospheric dispersion from sources also plays a critical role in controlling the regional distribution of naphthalene, leading to the widespread distributions noted in Figure 18. The high degree of spatial variability suggests one needs a multi-station network like those used to characterize ozone and NO_2 in Southern California to properly characterize naphthalene (and other PAHs) in urbanized regions.

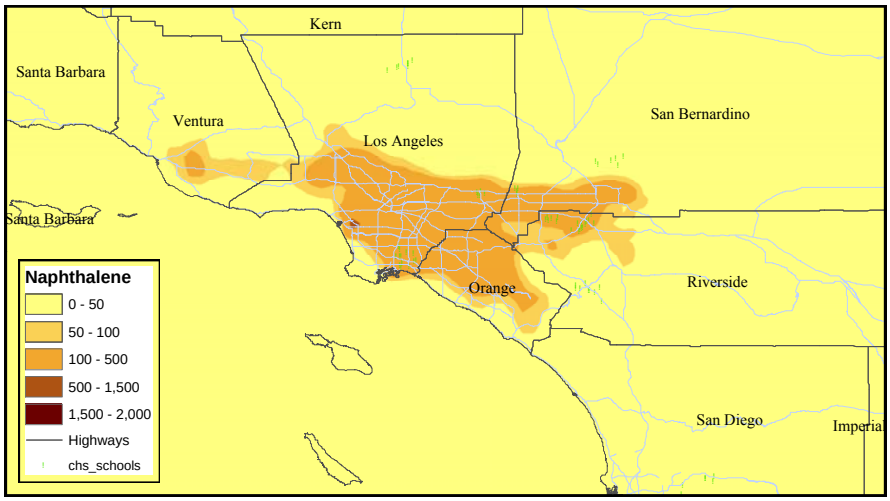

Figure 18. Predicted Distributions of Naphthalene Across the LAB Corresponding to 1998 Summer Emissions (in Units of ng/m^3)

The distribution of 1,4-naphthoquinone in both gaseous and aerosol phases generated by naphthalene photooxidation is shown in Figure 19. Significant differences can be seen between the concentration patterns of the primary and secondary compounds—naphthalene and naphthoquinone. The 1,4-naphthoquinone is concentrated inland along the mountain slopes where air trajectories arrive from the coastal region.

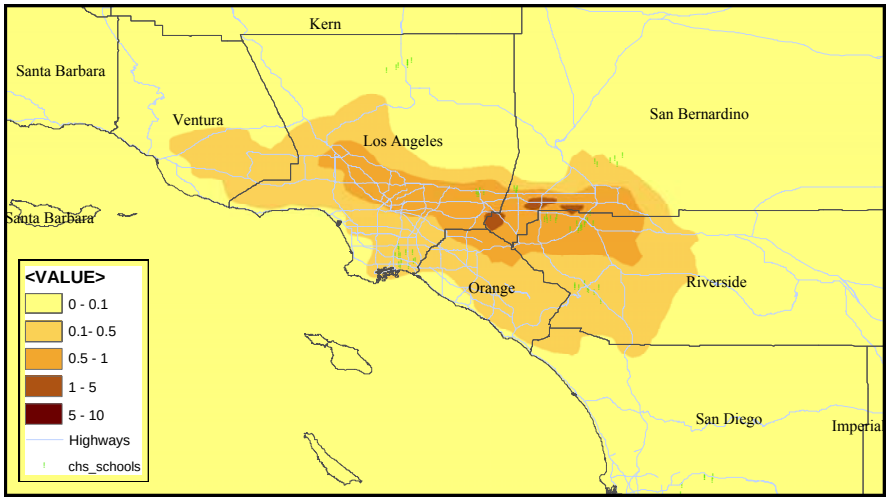

Figure 19. Concentration Contours for the 1,4 Naphthoquinone Byproduct of Naphthalene (in Units of ng/m^3)

In this analysis, 1,4-naphthoquinone concentrations are from photochemical production only. However, other processes not accounted for in this analysis may also contribute to the total burden. For example, the photo-transformation of naphthalene on solid surfaces and fly ash apparently generates naphthoquinones (Guillard, et al., 1993). Similar processes may occur on aerosol surfaces in polluted airsheds. Bunce, et al. (1997) noted the formation of naphthoquinones in the reaction of naphthalene with OH radicals and subsequent secondary reactions with a peak yield of 6% under their experimental conditions. Moreover, quinones may be emitted in vehicle exhaust (Choudhury, 1982). Further, the toxicities and health impacts of naphthalene oxidation products other than naphthoquinone, including naphthols, formylcinnaldehyde, and certain dicarbonyls, are largely unknown. The yield of each of these compounds from the oxidation of naphthalene ranges between ~3% and 35% (Sasaki, et al., 1997). It follows that the sum of these additional compounds could significantly exceed the predicted concentrations of 1,4-naphthoquinone based on the photochemical scheme in Figure 17. Further work is required to quantify the abundances of quinones in urban air, and to characterize their regional distributions and human exposure.

In the case of naphthalene, simulations and observations are in relatively good agreement. This implies that the total emission inventory may be within the range of a factor of 2. However, uncertainties about specific sources are larger, especially from mobile sources and fuel leakages. The demonstrated existence of distinct distribution patterns for the parent species and its secondary compounds (e.g., comparing Figures 18 and 19) implies distinct chemical "fingerprints"—consisting of combinations of spatial patterns and ratios of concentrations—that could be exploited to identify the contributions from individual sources (e.g., gasoline vaporization). This requires more detailed measurements, however, and cannot be pursued at this time.

Naphthalene Exposure Assessment with the REHEX Model. The naphthalene concentration estimates obtained from the SMOG model are employed in the Regional Human Exposure (REHEX) model to calculate population exposure statistics. Results show average hourly naphthalene exposures in Southern California under summer and winter conditions of 270 ng m^{-3} and 430 ng m^{-3}, respectively. Exposure to significantly higher concentrations may occur for individuals close to local sources, or in naphthalene "hotspots" revealed by simulations and observations. Such levels of naphthalene exposure may be used to gauge the potential health impacts of long-term naphthalene exposure. Results are also given for the distributions of 1,4-naphthoquinone, a naphthalene reaction product that may have significant health effects.

Application of the SMOG Model in Support of the SCPCS CHS

We have continued to improve and apply the SMOG model calculations to support the SCPCS CHS analysis being carried out by Winer, Lurmann and Wu, as summarized in the exposure modeling section of the report. New sources of emissions data have been incorporated in as a means of improving the overall reliability of the air quality and exposure calculations. The airshed model predictions provided background pollutant concentrations to estimate the exposure of individuals based on an analysis with the personal exposure model, IEM.

Updated Emission Inventories. To improve the predictions of particulate pollutant distributions for exposure assessment in the SoCAB, we updated primary emission inventories, incorporated a secondary organic aerosol (SOA) module, and integrated a biogenic emission database into the SMOG model. The latest emission inventory developed for the AQMD 2003 Air Quality Management Plan (AQMP) was adopted to estimate CO, NO_x, SO_x, VOC and PM emissions in the SoCAB. The 2003 AQMP emission inventory treats point, area, off-road and on-road sources. The on-road emissions were estimated using the CARB EMFAC-2002 emission factors, and transportation activity data developed by the Southern California Association of Governments (SCAG) in their 2001 Regional Transportation Plan. Emissions from off-road vehicles, including trains, ships, construction equipment, and utility engines, were determined using estimated activity levels and emission factors for these sources. The revised inventory was spatially distributed over a grid system composed of 5-km by 5-km grid cells spanning Southern California. The incorporation of this database reduced uncertainties associated with the older "standard" emissions, which were based on inventories constructed in 1997. With the new emissions database, we have performed SMOG simulations for periods corresponding to specific SCPCS field campaigns, and compared SMOG predictions against these data to check the fidelity of the model with the latest emissions.

Secondary Organic Aerosol (SOA) Module and Biogenic Emissions. SOAs are an important component of the total particulate burden in the LAB, and many of the SOA constituents are semi-volatile. Hence, the SOA module tracks production of condensable gases formed during the oxidation of VOC precursors, calculates the equilibrium distribution between the gas and aerosol phase, and predicts changes in aerosol size distribution due to the condensation/evaporation process. Emissions of SOA precursors from biogenic sources contribute to the aerosol organic component. Biogenic emissions calculated using the CARB's BEIGIS model has been implemented in SMOG. BEIGIS is based on a California-specific input database with a minimum spatial resolution of 1 square km and hourly temporal resolution. The enhancements to the SMOG model described above have significantly improved our ability to model the distributions and organic composition of particulates and their precursors in the SoCAB.

CHS Simulations. The updated emission inventories also allow more reliable simulations of criteria pollutants of interest to the CHS supported by the SCPCS. Detailed baseline calculations of the background pollutant levels at CHS locales have been employed in the IEM (Wu, et al., 2005a), supported by the SCPCS for epidemiological evaluation of the CHS cohorts. A series of SMOG simulations for specific episode conditions have been provided to the IEM team, including a detailed quantification of the spatially resolved background abundances for use with theCALINE4 model. The exposure of individuals has been analyzed with the personal exposure model with predictions of regional-scale gas and particle distributions and time variations associated with transported and local non-mobile sources corresponding to each of the CHS study areas. The SMOG background predictions for different seasons and sub-regions have been combined with roadway pollutant simulations with the CALINE4 model to provide the most detailed characterization to date of childhood exposure to air pollution in the SoCAB.

Modeling UF Near Major Roadways for Human Exposure Assessment

UF are suspected as causative agents for a wide range of adverse health outcomes including morbidity and mortality. Due to the fact that they contribute very little mass to C or F which EPA regulates, it is important to investigate UF properties and to quantify their concentrations and human exposure. Although the U.S. EPA currently regulates total particle mass below 2.5 micrometers in diameter, UFs represent the smallest particles in this range, with the largest concentrations and potential health impacts. In fact, $PM_{2.5}$ mass is not really an indicator of UF properties. These concerns have led European agencies to contemplate regulating particles by number rather than by mass. Taking into account the potential health effects of the UF and the fact that they contribute very little to C or F, it is important to investigate their properties and to quantify their concentrations and human exposure.

We developed and applied new modeling tools to characterize UF near major roadways. A new microphyical analytical parameterization in addition to the comprehensive microphysical treatment in SMOG were utilized. Field-measurement datasets were employed to test and validate model predictions near roadways. The CALINE 4 roadway line source code was used to achieve high spatial resolution. The emission rates of UFs from major roadways were estimated from field measurements and traffic counts of gasoline and diesel vehicles. A box-version of the SMOG air pollution model (Lu, et al., 1997a) with an explicit treatment of coagulation, condensation/evaporation, deposition, as well as dilution processes, was used to simulate the evolution of UFs as they move downwind from selected roadways. Preliminary simulations indicated that the model reproduced the main observed features of UF transformations near freeways. These detailed simulations also revealed the primary transformation processes that control UF number concentration and size distribution. These insights guided the development of a simplified UF analytical model that is practical for human exposure assessment applications.

Analytical solutions of the equations describing aerosol coagulation and vapor condensation in an expanding plume have previously been derived and used to simulate aerosol plumes from aircraft emissions (Turco and Yu, 1998; 1999). Similar techniques were adapted here to study freeway line sources of interest (e.g., Figure 20). The analytical roadway UF model accounts for the coagulation and condensational growth of a size distribution of aerosols from a specified source. The condensation processes that can be accounted for include volume condensation (or bulk reaction controlled uptake), surface-controlled condensation (typically applicable to very small particles), and diffusion-limited condensation (for larger particles). The UF parameterization yields the properties of UF evolving in traffic line emission plumes. The expansion rates of such plumes were calculated using measured (or in some cases predicted) CO concentrations. With initial UF number and mass concentrations, as well as estimates of the concentrations of condensing vapors, the particle number concentration and size distribution at downwind receptor points could be calculated. The simple UF model has been coupled with the CALINE 4 roadway line source model to determine UF distributions near major roadways due to vehicle emissions (Figure 20).

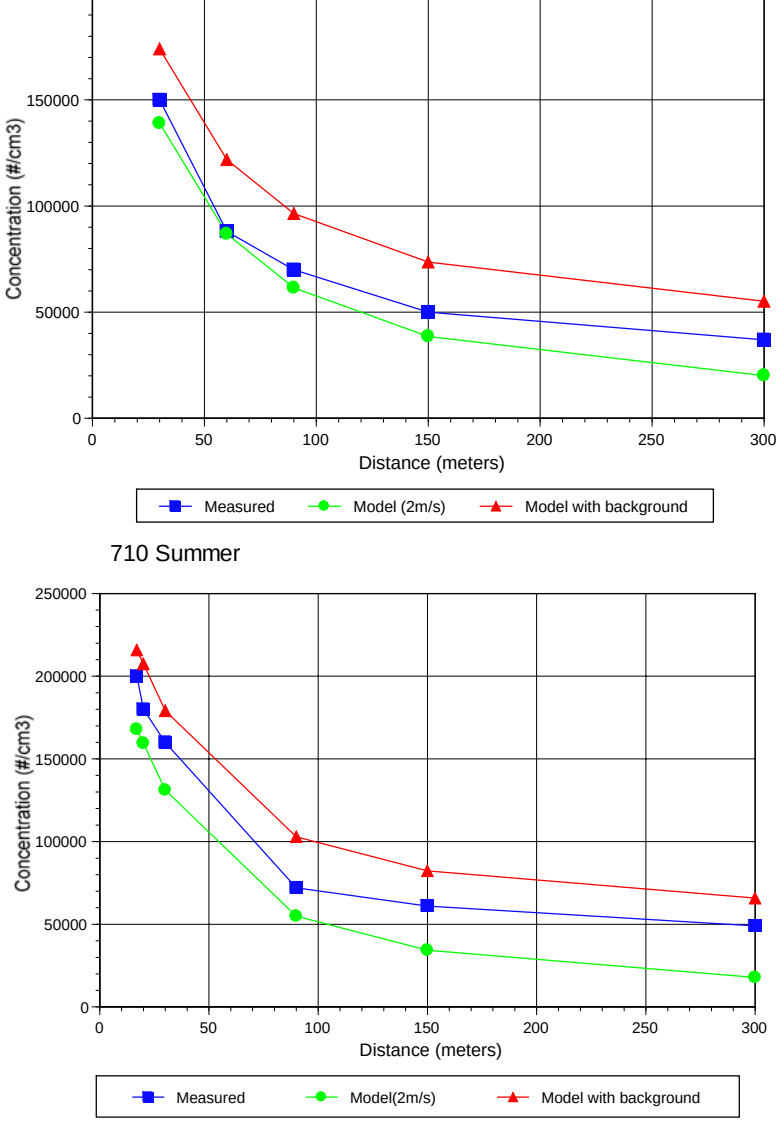

Figure 20. Comparisons of Observed (blue symbols) and Modeled UF Concentrations at the 405 and 710 Freeways

UF particle concentrations were predicted based on a microphysical parameterization in the CALINE 4 model (see the text). Circles and triangles show modeled concentration without and with background particles, respectively.

Motor vehicle exhaust is the major source of UF in urban environments. High particle number concentrations in the vicinity of freeways raise concerns regarding adverse health effects. However, field measurements show that UF number concentrations decline dramatically—and the corresponding size distribution changes rapidly—with increasing distance from major roadways (Zhu, et al., 2002a; 2002b; 2004). Comparisons of UF concentrations simulated with CALINE 4 and a UF parameterization are compared with measurements downwind of the 405 and 710 freeways in Los Angeles in Figure 20. The comparisons show that the modeling approach can provide useful information for exposure assessment under a variety of conditions that would be difficult to characterize observationally.

Conclusions

During the project period, significant accomplishments have been achieved in many areas, including the modeling of distributions of particle-borne materials and gaseous pollutants across the LAB, the assessment of the regional distributions of and human exposure to naphthalene and naphthoquinones in the LAB, application of the SMOG model in support of the CHS, and development of a detailed model for UF size distributions for freeway exposure studies. These achievements addressed the key National Research Council (NRC) research priorities of the use of advanced modeling to relate specific sources of PM to human exposure and the application of such models to connect toxicologically important constituents of PM to individuals and regional populations.

A new air quality and exposure modeling system, including a regional air quality model (SMOG), a local-scale line source model (CALINE4), an IEM and a population exposure model (REHEX), calculates individual or population exposures to vehicle-related pollutants by integrating pollutant concentrations in a variety of microenvironments, people's time-activity patterns, and ambient pollutant concentration estimates at different spatial resolutions (e.g. a point, tens of meters, or 5 km). This modeling system has facilitated analyses of exposure to local mobile emissions, non-mobile sources, and pollutants transported from upwind regions: these sources had not been investigated simultaneously and appropriately in previous studies. This advanced modeling system provides exposure assessment support for several projects conducted by the SCPCS and CHS, including the intra-community exposure variability analyses in the CHS study, the traffic density and emission assessment of the SCPCS, and investigation of exposure to species identified as of toxicological interest by SCPCS researchers. This system can also relate specific sources of pollutants to human exposure; quantify exposure of sub-populations that are potentially most susceptible to adverse health outcomes from PM-related pollution; create a linkage between the science resulting from the SCPCS/CHS programs and the policy and standard-setting goals established by the EPA; and aid in the design of cost-effective and optimally health-protective emission control strategies for F and particle-associated species.

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Project 16: Particle Dosimetry (R827352C016)
Investigators: Robert Phalen, Michael Oldham

Objective(s) of the Research Project: The Dosimetry Core had two objectives; it was a service and research core. Dosimetry, or the quantification of deposited and/or uncleared particulate

mass, is of importance in both the design and interpretation of the Center's epidemiology and toxicology studies, and for estimating population exposures using air-monitoring data. Two major aspects of dosimetry are: (1) determining the initial amounts of pollutant deposited on specific sites within the respiratory tract, and (2) determining the fates of deposited material with respect to retention, movement and bioavailability. The Dosimetry Core initiated original research to improve relevant dosimetry models and collaborated with other investigators to improve the design, interpretation and impact of their research.

Achieving these goals required maintaining several important assets, including operational computer codes; an appropriate library and literature data base; a qualified computational technician; and hardware for running codes, printing results and preparing publication-quality figures, tables and charts. The Dosimetry Core also initiated a workshop in order to support and guide Center research and to provide coordination with PM-related dosimetry activities outside of the Center.

Summary of Findings:

Year 1

The Dosimetry Core was active in several areas including:

1. Acquiring and installing dosimetry software that is applicable to adults, children and some laboratory animals (rat, mouse, and ferret)
2. Distributing software and software documentation to Center investigators, and providing tutorials on obtaining and using the output

3. Helping Center postdoctoral researchers Dr. Jacques and Dr. Yu refine their research on human dose measurements, and bioavailability modeling

4. Helping doctoral student Mr. Oldham with his thesis research on validation of Computational Fluid Dynamic (CFD) particle deposition models

5. Developing plans for a dosimetry workshop

6. Presenting scientific talks on particulate air pollution, including a dosimetry talk at the UCLA Center's first workshop

The acquired dosimetry software included that generated by 1) the National Council on Radiation Protection and Measurements (NCRP), 2) the International Commission on Radiation Protection (ICRP, the software is LUDEP), 3) the Chemical Industry Institute of Toxicology/National Institute of Public Health and the Environment of the Netherlands (CIIT/RIVM, the software is MPPDep), 4) the University of California Irvine (UCI, based on NCRP software), and 5) Fluent Corporation (the CFD software is called FIDAP). Each software package was tested and is functional. Each package was found to have unique advantages and limitations. The NCRP model includes adults and children of all ages, provides local deposition doses generation-by-generation, and is the most modifiable (in terms of anatomical data input)

for research purposes. The ICRP software includes children and women, and it tracks the transport of inhaled particles leaving the lung, but it does not provide local generation-by-generation doses, and it is not easily manipulated to model individuals. The CIIT/RIVM software, MPPDep (Multiple Path Particle Deposition Model), which became available in July 1999, includes adult males and the laboratory rat. MPPDep calculates particle deposition on both a lobe-by-lobe and generation-by-generation basis, inhalability is an option, ventilation and exposure duration can be specified, and it produces publication-quality graphical output. However, MPPDep is not easy to modify to model individual lung anatomies. The University of California, Irvine (UCI) model is an evolving extension of the original NCRP code. We have expanded the code to include hygroscopic aerosols, tobacco smokes and additional species of laboratory animals (rats, mice and ferrets). This model is our main research tool for improving dosimetry models. The FIDAP software is a very-advanced CFD program that runs on our parallel processor. Specialized training is essential for FIDAP users. This package is capable of solving complex-geometry airflow fields and then tracking individual particles through the geometries. FIDAP has been installed, tested and used to solve flow fields in a 3-generation airway branch that exactly matches physiologically-realistic hollow models in our laboratory. We have performed several particle deposition calculations and compared these predictions with actual particle depositions using monodisperse particles in the hollow laboratory models. Initial results were encouraging in that the computed particle depositions matched the observed patterns, thus holding the promise of eventually providing microdosimetric information at specific sites in the respiratory tract. However, the FIDAP code failed to predict the observed total particle deposition as seen in hollow models.

Collaborations with Center Investigators. Dr. Peter Jacques, who was involved in human clinical exposures, worked with us to plan measurements of particle deposited dose in individual subjects during exposures to CAPs. If this could be achieved, the responses of individuals can be correlated with their measured total, and calculated regional particle depositions. In addition, Dr. Rong Chun Yu worked with us to define his research on the bioavailability of organics absorbed in or adsorbed on inhaled F. We helped to define a generic "atmosphere to target tissue" research model that could then be applied to specific cases, such as organic-coated diesel exhaust particles.

Our involvement in Mr. Oldham's doctoral research on CFD modeling helped him to focus on particles of importance in urban air pollution in the 1 to 10 μm aerodynamic diameter size range. Because CFD models are the only ones capable of computing individual particle deposition locations, as opposed to "smeared" doses, they may be able to explain differences between normal and susceptible subpopulations, if such differences are due to anatomical abnormalities that produce hot spots of particle deposition. Mr. Oldham also worked on converting clinical MRI (Magnetic Resonance Imaging) scans on individuals into forms that can be input into FIDAP for flow solutions and particle deposition calculations. If this effort is successful it will be especially useful for evaluating particle deposition in the upper airways of children and adults with respiratory diseases.

<u>Year 2</u>

The following lists some of the Year 2 accomplishments:

1. Plans were made with Dr. Kleinman for performing a respiratory-tract morphometric and dosimetric study on the Balb/c freeway-study mice. A preliminary protocol and study design were developed.

2. All dosimetry software was maintained in a current and functional state. This included renewal of the Computational Fluid Dynamics software and installation of upgrades. Extraction of hourly wind, temperature, dew point and visibility data for 13 weather stations in the L.A. Basin was added as a new capability.

3. Plans for an international conference on particulate material were initiated. The American Association for Aerosol Research (AAAR) took the organizational lead, and the U.S. EPA contributed start-up funds. The Dosimetry Core was centrally involved in directing this important conference, the fourth in a series started in 1994.

4. Our graduate student, Mr. Oldham completed the experimental portion of his doctoral research on CFD model validation for PM deposition and began writing his thesis. The STAR grant will be acknowledged in journal publications derived from his thesis. It appears that the CFD approach is promising for defining local regions in airways that have high particle depositions. The computed patterns of deposition predicted for 1, 3 and 10 μm aerodynamic diameter particles were consistent with his laboratory experiments. Mr. Oldham shifted his main focus to conducting the rodent exposure "freeway study" with Drs. Kleinman and Sioutas.

5. At every opportunity, dosimetry discussions occurred between the Dosimetry Core and other SCPCS investigators. Topics included considering body size factors as dose modifiers in the "children's study," performing Balb/c lung morphometry, defining the air-to-lung PM transfer coefficient for mice in exposure cages used in the freeway study, and upgrading the wind data during exposures of mice to CAPs.

Year 3

The following list highlights our accomplishments:

1. The lung morphometry on the Balb/C freeway study mice was completed. This involved making about 2 dozen in-situ lung casts, selecting 3 casts from ovalbumin sensitized mice and 3 casts from normal mice for detailed morphometric measurements, performing the measurements, and using them to calculate particle deposition efficiencies. In addition, 20 casts were subjected to measurements of a few airways in order to determine the extent to which variations in casting volume influenced the cast airway sizes. The results were interesting. First, the ovalbumin sensitization did not significantly alter airway dimensions. This means that sensitization is not expected to change the deposition of particles in the freeway study, provided that sensitization does not significantly alter breathing patterns during exposure to CAPs. Second, variations in casting volume (which are unavoidable due to the tiny cast volumes used in mice) did not significantly change the sizes of airways. Third, the morphometric measurements of the Balb/C mice tracheobronchial trees were

significantly different from those obtained by us previously on $B6C3F_1$ mice. This is a novel finding that implies the strain of mouse will significantly influence its deposition dose in an inhalation study. In past dosimetry work, "a mouse was a mouse," but in the future, the mouse type/strain will also be seen as an important piece of dosimetry information. These findings were significant enough to trigger a paper.

2. Our doctoral student, Michael Oldham, completed his program. His research involved validation of a Computational Fluid Dynamic particle deposition model by comparing predictions to bench-top particle depositions in hollow airway models. The thesis, "Comparison of CFD Predictions and Experimental Results for Local Particle Deposition Patterns in Idealized Human Airways," supports the use of high concentrations of PM in Dr. Nel's SCPCS research project.

3. A dosimetry workshop was held at UCLA on October 26, 2001. The workshop served to bring several SCPCS investigators together to integrate their knowledge on Dosimetric issues related to several of the Center's themes. Presentations by Drs. Sioutas, Cho, Hinds, Phalen, Yu and Nel were followed by focused discussions. A significant result from the workshop was that the elevated PM concentrations used in Dr. Nel's in-vitro research could be supported as being realistic representations of local doses in human lung regions during actual exposures in the L.A. Basin as defined by Dr. Hinds' research. This result is a useful scientific advance, and it also strengthens future publications of Dr. Nel. The workshop also had some implications for the SCPCS epidemiology research in that the importance of understanding "individual" exposures was emphasized. The workshop results were used to contribute to the plans for seeking renewal of the STAR Grant program.

4. A transfer coefficient study was started with nine mice being exposed to 1 μm diameter fluorescent tracer particles in the freeway study animal exposure system. The purpose was to quantify deposition in the mice under exposure conditions. Additional tracer particle sizes were planned for Year 4.

Year 4

We contributed heavily to the freeway study, including helping with logistics, conducting exposures, performing bioassays, and characterizing the exposure system's delivery of air pollutants to the mice. Support was provided to Dr. Harkema's group (from Michigan State University) by helping with lung casting of the Brown Norway rat. We also helped neurotoxicologist, Dr. Campbell of UCI to get neurotoxicity data on mice exposed in the freeway study. With respect to research, we worked on how mouse variety can significantly affect the deposition of inhaled particles.

Year 5

This grant year was productive in several ways. The objectives included publication of research results, continuing support of the "freeway study" and preparation and measurement of rodent lung casts in order to produce data on particle doses in toxicology studies. The Dr. Nel paper for which we provided dosimetry-related contributions was published (Li N, Hao M, Phalen RF,

Hinds WC, Nel AE. Particulate air pollutants and asthma: a paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clinical Immunology* 2003;109:250-265). In support of the Freeway Studies, we worked on the performance of the mouse exposure system that is supplied with air pollutants by the particle concentrator.

During Year 5 the Dosimetry Core continued to support the freeway studies by assisting during exposures, endpoint acquisition and data analysis.

The preparation and morphometric analysis of lung casts continued throughout the year. Ten rat lungs were cast and stored for measurement, and large-airway morphometry was conducted on casts made in Brown Norway Rats. We concluded that this rat, which is widely used in PM studies, appears to have normal (for rats) large airway structure. Arrangements were discussed with the CIIT Centers for Health Research for incorporating our rodent lung measurements into their widely used dosimetry software MPPD1.

Year 6

The final year focused on publications and presentations.

Conclusions

1. The aerosol dosimetry software packages (LUDEP and MPPD) in current use that predict inhaled particle deposition efficiencies are both useful for predicting human doses. Although each program gives slightly different values for similar inputs, the differences are within the range of values expected in human populations. Because MPPD1 (the latest version) is free (from the CIIT website), user-friendly and includes adults, children and rats, it is recommended for use in the SCPC. However, LUDEP is also very acceptable.

2. Computational Fluid Dynamic approaches to solving inhaled particle deposition problems have great promise in that individual differences and micro deposition patterns can be addressed. Although such approaches are not adequately validated for use in epidemiology research, they are useful for designing in-vitro particle toxicology studies.

3. Our morphometry and particle deposition calculations indicate that different mouse strains/varieties can receive different particle doses, even when exposed to the same air pollutant. Thus, dosimetry information in one mouse strain does not necessarily apply to another strain.

4. Ovalbumin sensitization of Balb/C mice did not significantly change airway dimensions, which simplifies interpretation of particle studies in which sensitized and non-sensitized animals are compared.

5. Sophisticated dosimetry calculations support the use of relatively large particle doses in in-vitro studies, if particle deposition hot-spots in the lung are believed to relate to effects.

6. Whole-body aerosol exposures of mice can provide for efficient inhalation of CAPs. However, careful design and testing of exposure cages is required.

7. The current state of modeling inhaled particle deposition in children is strong. Predictions using computer software models are consistent with clinical-setting measurements.

8. Concentrated airborne particulate matter exposures appear to be capable of increasing inflammatory endpoints in the brains of Balb/C mice (Campbell et al., 2005).

References:

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Li N, Hao M, Phalen RF, Hinds WC, Nel AE. Particulate air pollutants and asthma: a paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clinical Immunology* 2003;109:250-265.

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Relevant Web Sites: http://www.scpcs.ucla.edu